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[GANN, Vol. 41; March, 1950]

STUDIES ON THE NUMBER OF CELLS NECESSARY FOR THE TRANSPLANTATION OF YOSHIDA SARCOMA

(Transmission of the tumor with a single cell)
(With Pl. I-II)

KAKUZO ISHIBASHI
Department of Pathology, Tohoku University, Sendai
(Director: Prof. T. Yoshida).

INTRODUCTION

When rats suffering from Yoshida sarcoma (1, 2, 3) come near the last stage, tumor cells are occasionally found in their circulating blood. I first made the transplantation tests with the blood of tumor animals in the most advanced stage and obtained positive results in the greater number of the cases. By finding the percentage of tumor cells to leukocytes in the blood smear, I calculated, from the amount of the injected blood, the number of tumor cells implanted, the average number of leukocytes contained in 1 cmm of blood being computed at 10,000. In this way it was estimated that about 8,000 tumor cells were enough to bring about the positive result, as is shown in Table 1.

In our laboratory about 0.01 cc of tumor ascites, in which from 1 to 10 million tumor cells are to be found, is injected in the routine transplantation of Yoshida sarcoma. Compared with this number, 8,000 cells in the blood inoculation may seem remarkably small, and this suggested to me the question of what is the minimum number of tumor cells necessary for the transplantation of Yoshide sarcoma.

Numerous experiments have been performed to find the least number of cells necessary for the transplantation of tumors. As for ordinary tumors, Costa (4) said, for example, that at least, 50,000 tumor cells were necessary while Witte (5) expressed his view that 1 million cells were not enough to ensue a successful result. Morimoto (6) stated that over a certain number of tumor cells were always required, when Honda's rat sarcoma was transplanted, though he did not give any concrete figure. On the other hand, Furth and Kahn (7) reported a success in transplantation with a single cell in their studies on the transplantable leukemia of mice, which is presenting a neoplastic character.

In all these experiments the cell suspension obtained by grinding or cutting up a piece of tumor tissue was used. But I made systematic experiments with Yoshida sarcoma, because it was believed to be the most suitable material for this kind of study, with various advantages:— i) it is originally a fluid

neoplasm, whose cells proliferate in a state of suspension in peritoneal cavity, ii) the transplantability is so high as 98 percent, iii) the conditions of the tumor can be examined very easily at any time required after the inoculation in the smear of the ascites, etc.

In addition, the question of how many cells are required for the transplantation of tumors is related to the question of whether tumors can be transplanted without any tumor cell. These are the face and the back of one and the same question. So, I also tried at the same time to examine if tumors could be transplanted without any tangible or visible tumor cell.

Experiments

I. Transplantation with the estimated number of tumor cells.

Material: In any and every case of the following experiments I used tumor ascites of Yoshida sarcoma. This ascites was obtained from tumor rats more than five days after the usual transplantation when tumor cells had proliferated in their peritoneal cavities, presenting the state of pure culture of tumor cells. The ascites taken from tumor rats was diluted with various solutions before a fixed amount of it (0.1 cc-0.01 cc) was inoculated into the peritoneal cavity of normal rats weighing 100 g or so.

Method: As it was known that 8,000 tumor cells were enough to bring about the positive result through the above-mentioned tests by using blood, I carried on my further experiments with smaller numbers of tumor cells.

The tumor ascites mentioned above was prepared and the tumor cells contained in it were counted in the blood counting chamber; dilutions were made by ten times successively with physiologic NaCl solution for the inoculation of small numbers of cells. Then 0.1 cc of each diluted ascites was inoculated into the peritoneal cavity of respective normal rats with a pipette which H. Sato (8) had designed for this purpose.

In these cases the actual number of cells inoculated was estimated by calculation.

Result: As is shown in Table 2, only 20 tumor cells caused the positive result.

It was remarkable that the time required for the growth of the tumor was prolonged to a great extent when a small number of cells were transplanted. In the usual transplantation, it takes three to four days for the tumor cells to sufficiently proliferate to present the state of pure culture in the peritoneal cavity, but the time was prolonged to seven days in case the number of inoculated cells was lowered to 30,000, and the time was prolonged to 18 and 16 days when the number was lowered to 8,000 and 2,000 respectively (Tables 1, 2).

This period, however, is prolonged with a certain limit. There can be little difference found between the inoculations of 20 cells (14 days) and 2,000 cells (16 days). This leads to the conclusion that the limit may be placed at two or three weeks even to an extremely small number of cells.

II. Transplantation with tumor cells actually counted

(A) Case in which tumor ascites was diluted with Tyrode solution.

Method: As I knew, by the practical tests referred to above, that such an extremely small number as twenty of tumor cells, though roughly estimated, were enough to bear the positive result, I tried the following experiments to actually count the tumor cells to be inoculated.

1) A micropipette (Fig. 1), made of a glass tube and bent in the end at a right angle, was first fixed on the micromanipulator (Fig. 2). Next, its tip was brought in the center of the visual field under the microscope, at the upper surface of the moistchamber attached to the micromanipulator, then was let down slightly.

2) A cover glass, on which several droplets of Tyrode solution were deposited, was bridged over the moist-chamber with the droplets hanging. Then the droplets were brought in the center of the visual field under the microscope and were sucked into the micropipette whose tip had been moved upwards by the aid of the micromanipulator. This tip was let down slightly again.

3) When some droplets of the tumor ascites, which had been diluted 50,000–100,000 times with Tyrode solution, were placed on a cover glass and were brought under the microscope just as in the same manner as mentioned above, the tumor cells contained in a droplet were possible to be counted one by one (Fig. 3). After an actual count of the tumor cells in them had been made, the droplets were put into the micropipette whose tip had been moved upwards by the micromanipulator. This tip was let down slightly again.

4) Several droplets of Tyrode solution were sucked again, in the same way as referred to in 2) above, into the micropipette to the total amount of 0.01 cc. Next the micropipette was removed from the micromanipulator and after its hooked portion was broken off, the whole content was injected into the peritoneal cavity of a normal rat.

It would be clear that in this case the number of cells inoculated was not larger than that arrived at by actual counting. And the tumor ascites actually inoculated might be a solution diluted 1 to 5 million times at its highest dilution, because the original tumor ascites had first been diluted 50,000–100,000 times, then again several times to a few ten times.

Result: As is shown in Table 3, the positive result was effected again with 20 inoculated cells. But 8, 6, 3 and 1 cell inoculations turned out negative in all the cases.

Twenty may be remarkably small as the number of cells required for the transplantation of tumors. Although Furth and Kahn reported a successful transplantation with a single cell, they secured the positive result in only 3 cases out of 65 experiments, in which they used Tyrode solution after adding mouse serum to it as the suspension medium. In other experiments, however, where only Tyrode solution was used, the result was unfavourable, i. e. in the ten cases of 14-100 cell inoculations they had always the negative result.

If this fact is taken into consideration, the successful result which 20 cell inoculation produced in two cases of the two experiments with only Tyrode solution may be regarded as a new contribution to the transplantation of tumors.

The inoculation of less than 10 tumor cells produced a fruitless result in every case of my six experiments, in which Tyrode solution was used simply. This, however, suggested the possible success even with a much smaller number of cells, if the peritoneal fluid of normal rats was added to this Tyrode solution or physiologic NaCl solution and so I tried the following experiments.

(B) Cases in which tumor ascites was diluted with physiologic NaCl solution added with normal peritoneal fluid.

Method: The same procedure as in (A) was followed ; only, as I have said, physiologic NaCl solution to which was added normal peritoneal fluid was used as the suspension medium.

This mixed solution was the supernatant liquor obtained by the following process. First I injected 15-20 cc of physiologic NaCl solution into the peritoneal cavity of a normal rat, then aspirated 5-7 cc of it after 30 minutes and centrifugated it for 60 minutes with the frequency of 3,000 cycles per minute. The supernatant liquor was thus obtained.

Result: As is shown in Table 4, the inoculations of 2 cells and one single cell were conducted with successful results.

In these cases, too, it required 17 and 15 days respectively for the tumor cells to proliferate in the peritoneal cavity in the state of pure culture. But no marked difference was found regarding the time required from 20 or 2,000 cell inoculation.

Since the fact was revealed that the inoculation of one single cell or two cells could bring about the positive result, the question would naturally be raised as to the possibility of transplantation without any tangible or visible tumor cells. I was thus driven into the next experiments.

III. Transplantation with ascites free from cells.

Method: Tumor ascites, which had been diluted 4-17 times with simple physiologic NaCl solution or with mixture of physiologic NaCl solution and normal peritoneal fluid, was centrifugated for 30 minutes (3,000 cycles) and 90 minutes (3,000 cycles), respectively, in order to obtain the cell-free supernatant liquor, of which several droplets were deposited on a cover glass just in the same manner as explained in (A) of Experiment II, and were examined under the microscope. About 20-30 droplets (0.01 cc in total amount)—strictly cellfree —were inspired into a micropipette by the aid of the micromanipulator. The micropipette was then removed from the micromanipulator and after its hooked portion was broken off, the whole content was injected into the peritoneal cavity of a normal rat.

For the sake of control a few droplets taken from the surface or sediment and containing a small number of cells, which had already been counted under the microscope, were injected into the peritoneal cavity of another normal rat, presenting always positive results.

Result: As is shown in Tables 5-9, the results in 15 cases were all negative with the exception of one. The course of the disease in this single positive case was quite analogous to that of 1 or 2 cell inoculation.

Microscopic Findings

1) In the case of 2,000 cell inoculation, a small number of tumor cells are found in the ascites after 24 hours. There are two kinds of these tumor cells; one is small and has a pycnotic nucleus, the other being relatively large and possessed of a monocytic appearance. The latter, however, is readily distinguished from a normal monocyte by the condition of cytoplasm and the form of nucleus. Among these cells, several figures of mitosis are observed about 5 days after. In this stage, the differentiation between a tumor cell and a large monocyte is rather difficult owing to the lowered basophilic character and the increased vacuolation of the cytoplasm of the tumor cell, but daily observation makes it possible. About 10 days after, however, in the stage of manifest proliferation, the tumor cell comes to be clearly distinguished from a normal monocyte by its increased basophilic character of the cytoplasm, its rounded and clear-cut cell-contour, and its hyperchromatic nucleus, etc. In addition, numerous figures of mitosis come into notice. Thereafter tumor cells proliferate more rapidly.

Through these stages, any transitional form is never demonstrated between normal cells and tumor cells.

2) In the case of about 20 cell incoulation, any identifiable element as a tumor cell can not be found for a long time after the inoculation. It is about 10 days after that an undeniable tumor cell is noticed in the ascites. Once it appears, it proliferates with considerable rapidity.

3) In relation to the virus problem it comes into question if normal cells in the peritoneal cavity (i. e. monocytes or lymphocytes) are able to proliferate with an ensuing malignant transformation into tumor cells in the course of the first 10 days or so. Any transitional form between normal cells and tumor cells has never been demonstrated in spite of constant care in every case (Fig. 4, 5).

4) In case that inoculations are made by a small number of tumor cells with ensuing negative results or by ascites free from cells, it is noticed that neutrophil leukocytes appear in the peritoneal fluid at first. They reach the maximum in number 2-4 hours after the inoculation, then decrease rapidly and at last almost all disappear 3 days later. Monocytes enter the stage a little later. They increase gradually following the decrease of neutrophil leukocytes. Hand in hand with the increase in number their bodies enlarge, presenting the appearance of macrophages, and in fact they phagocyte actively degenerated leukocytes, etc. These monocytes decrease rather sluggishly and usually go back to the normal state about a week after. These changes should be regarded as the tissue reaction for a foreign body invading an animal, having no specific characters at all.

Discussion and Summary

1) First of all I must take the question of whether the transplantation of Yoshida sarcoma is caused by the tumor cell itself.

In the experiments with ascites free from cells, only one case out of 15 presents the positive result. Even a single case resulting in a success puts before us a question to be discussed—a question of whether there exists a certain extracellular oncogenic agent such as a virus in this tumor.

Supposing such an agent exists in this tumor, the fluid containing the more of it must present the higher transplantability. As a matter of fact, the tumor ascites diluted 4-17 times, in which a great deal of such an agent, if it exists, might presumably have been contained, bore the negative result with one exception in 15 cases, when tumor cells had been excluded from it; while, the tumor ascites diluted to such a high degree as 5 million times produced the positive result, when a very few tumor cells, even a single one, had been included in it.

If such an agent really exists, moreover, the transplantability of this tumor

can not be influenced by the number of the tumor cells, but, in fact, it markedly goes down according to the gradual decrease of the number of tumor cells.

From these facts, it is hardly to be believed that there exists any such extracellular oncogenic agent at all.

Besides, tumor cells were occasionally found in a droplet taken from the supernatant by the centrifugation for 60 minutes at 3,000 cycles. Although such a cell-containing droplet was omitted, absolute prevention from an entrance, among numerous droplets, might not always be expected. If once this droplet containing cells was inoculated into the peritoneal cavity, it would be an easy task for the cell to proliferate immediately, since 1 or 2 cell inoculation caused the positive result.

Furthermore, from the microscopic findings, the proliferation of tumor cells implanted was seen every time, while the picture of malignant transformation of normal cells in the peritoneal cavity could never be observed.

The course of the disease in the one positive case mentioned above (i. e. positive case in cell-free inoculation) was quite analogous to that of 1 or 2 cell inoculation.

I came thus to the conclusion that the transplantation of Yoshida sarcoma does not depend upon any extracellular oncogenic agent, but upon the proliferation of the implanted tumor cell itself.

2) Next I want to express my opinion on the minimal number of tumor cells necessary for the transplantation of Yoshida sarcoma. Table 10 is compiled, from the results of the transplantation with a small number of tumor cells. Although a successful transplantation could be expected even from one single tumor cell, the positive result was not attained in every case where less than 10 tumor cells were applied and even 23 or 30 cells produced the negative result.

These seem to be due to the following conceivable factors: not all cells used for inoculation were viable or capable of proliferation, one single cell sucked into the micropipette might happen to be a normal cell (monocyte or lymphocyte) occurring among tumor cells; furthermore, the cells once sucked into the micropipette might possibly remain there, failing to reach the peritoneal cavity of a rat, or they might be injured during the process of manipulation and fall into degeneration, though they reach the peritoneal cavity.

In this connection, it was remarkable that successful inoculations were made with such a very small number as 1, 2 or 11 of cells, when the tumor ascites was diluted with physiologic NaCl solution with peritoneal fluid of normal rats as an addition. In other words, it may be supposed that the solution added with peritoneal fluid would work less injury to the cells than plain Tyrode

solution or physiologic NaCl solution.

These results correspond with the report of Furth and Kahn that with Tyrode solution solely, 10 cases of 14-100 cell inoculation ended all in the negative result, while, with the one containing mouse serum, the transmission with a single cell was successful in 3 cases out of 65.

Yoshida sarcoma, as mentioned above, can be transplanted with a single cell though this inoculation may not always give a practical success. In fact, even 20-30 cell inoculation may sometimes be carried out with the negative result. But, as is shown in Table 10, the inoculation with a number larger than 32 of cells was positive in all cases. These facts make me confident that about 40-90 cells would be enough to bring about the positive result, taking into account various ill-effects possibly exercised on the tumor cells during the process of manipulation.

3) Finally I intend to state the course of proliferation of the tumor cells implanted. In the usual transplantation of Yoshida sarcoma, it requires 3-4 days for the tumor cells to proliferate in the peritoneal cavity, presenting the state of pure culture, while it takes much longer time in the transplantation of a small number of cells, that is, 7 days with 30,000 cells and more than 10 days with 8,000 to 2,000 cells. This period, however, is not prolonged without limit. Both 1 and 20 cell inoculations require 2 weeks or so for this purpose. The surviving days of tumor animals, after reaching the state of pure culture of tumor cells, are always the same both in the case of usual transplantation and in that of a small number of cells. The animals die of the tumor about a week after reaching the state of pure culture of tumor cells. In other words, the difference of the number of implanted cells between the usual transplantation and that of a small number of cells appears sensitively in the duration they require for growing up into the state of pure culture. The difference, however, is not always proportional to the value estimated mathematically. This result may be explained by supposing that all the implanted cells do not begin to proliferate in the same manner, but some of them go down in degeneration owing to the injury they receive during the procedure of manipulation or to the defensive power of the animals and so on.

Conclusion

Yoshida sarcoma is successfuly transmitted even with a single tumor cell, but without any tumor cells the transplantation is impossible.

The transplantation of Yoshida sarcoma is due only to the inoculation of living tumor cells.

Table 1. Transplantation by blood

No.	Amount of injected blood (cc)	Percentage of tumor cells to leukocytes in the blood	Number of tumor cells implanted (estimated)	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
1	0.4	0		−			
2	0.2	0		+	16	23	
3	0.5	0		−			
4	0.5	0		+	16	24	
5	0.3	1.0	30,000	+	7	8	
6	0.3	/		+	16	26	
7	0.5	/		+	23	29	
8	0.3	0.4	12,000	?			*
9	0.1	0.8	8,000	+	18	25	
10	0.1	0.8	8,000	?			**
11	0.05	2.5	12,500	+	11	15	
12	0.05	0		−			
13	0.05	0		−			
14	0.02	0		−			

* Died on 7th day.
** Died on 9th day.

Table 2. Cases in which the number of implanted tumor cells were estimated *

No.	Number of tumor cells implanted (estimated)	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
282	2,000	+	16	23	
281	200	+	22	32	
291	30	−			Retransplanted**on 91st day with ensuing positive result
292	30	−			Died on 65th day; no change in the abdominal cavity found

287	20	+	14	21	
288	2	—			Retransplanted**on 11lth day with ensuing negative result

* Tumor ascites was diluted by 10 times successively with physiologic NaCl solution.

** Retranspiantation was performed in the same manner as the usual transplantation.

Table 3. Cases in which the number of implanted tumor cells were actually counted (Diluted with Tyrode solution)

No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
233	20	+	15	21	
294	20	+			*
300	8	—			Retransplanted on 44th day with ensuing positive result
302	6	—			
301	3	—			
298	1	—			Retransplanted on 36th day with ensuing positive result
299	1	—			

* Undeniable tumor cells were observed on 11th day, increasing thereafter; died on 14 day.

Table 4. Cases in which the number of implanted tumor cells were actually counted (Diluted with physiologic NaCl solution added with normal peritoneal fluid

No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
339	1	+	15	25	
322	2	+	17	24	
338	4	—			Retransplanted on 68th day with ensuing positive result
336	5	—			

337	20	+	12	20	
331	48	+			*

* The case turned out to be a subcutaneous transplantation actually; with formation of a small hen-egg sized tumor within the abdominal wall: died on 40th day.

Table 5. Cases in which tumor ascites was diluted by 10 times with physiologic NaCl solution (Centrifugated for 30 min. at. 2,000 cycles)

	No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
	305	0	—			*
	306	0	—			**
	307	28	—			***
Control	308	61	+	14	21	
	309	****	+	6	14	

* Retransplanted on 46th day with ensuing positive result.
** Diet on 107th day, no change in the abdominal cavity found.
*** Retransplanted on 43rd day with ensuing positive result.
**** 0.02 cc of the 10 times diluted ascites.

Table 6. Cases in which tumor ascites was diluted by 4 times with physiologic NaCl solution added with normal peritoneal fluid (Centrifugated for 30 min. at 2,000 cycles)

	No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
	310	0	—			*
	311	0	—			
	312	0	—			**
Control	313	11	+	14	22	
	307	***	+	25	33	****

* Retransplanted on 99th day with ensuing positive result.

** Retransplanted on 35th day with ensuing positive result.
*** 0.01 cc of the 4 times diluted ascites.
**** The case turned out to be a subcutaneous transplantation. Later, tumor cells were seen in the peritoneal cavity.

Table 7. Cases in which tumor ascites was diluted by 17 times with physiologic NaCl solution (Centrifugated for 60 min. at 3,000 cycles)

	No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
	314	0	—			*
	315	0	+	16	23	
	316	0	—			**
	317	0	—			*
	318	0	—			**
Control	320	5	?			***
	319	****	+	11	24	

* Retransplanted on 41st day with ensuing positive result.
** Retransplanted on 47th day with ensuing positive result.
*** Died on 7th day.
**** 0.01 cc of the 17 times diluted ascites.

Table 8. Cases in which tumor ascites was diluted by 6 times with physiologic NaCl solution (Centrifugated for 90 min. at 3,000 cycles)

	No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
	330	0	—			*
	331	0	—			**
	326	4	—			***
Control	327	58	+	14	17	
	316	****	+	5	10	

* Retransplanted on 21st day with ensuing positive result.

** Retransplanted on 24th day with ensuing positive result.
*** Diet on 20th day; no change in the abdominal cavity found.
**** 0.02 cc of the 6 times diluted ascites.

Table 9. Cases in which tumor ascites was diluted by 5 times with physiologic NaCl solution (Centrifugated for 90 min. at 3,000 cycles)

	No.	Number of tumor cells implanted	Result	Time needed for reaching the state of pure culture in days	Length of life in days	Remarks
	332	0	—			*
	333	0	—			
	335	0	—			**
Control	334	32	+	13	22	
	330	***	+	7	16	

* Retransplanted on 109th day with ensuing positive result.
** Retransplanted on 43rd day with ensuing positive result.
*** 0.01 cc of the 5 times diluted ascites.

Table 10. A general view of a few cell inoculation.

Number of tumor cells implanted	Number of rats injected	Number of successful injections	Length of life in days *
2,000	1	1	23
200	1	1	32
61	1	1	21
58	1	1	17
48	1	1	**
32	1	1	22
30	2	0	
28	1	0	
20	4	4	20–21 (21)
11	1	1	22
8	1	0	
6	1	0	
5	2	0	
4	2	0	
3	1	0	
2	2	1	24
1	3	1	25

* The figure in parentheses gives the average.
** The case turned out to be a subcutaneous transplantation actually; died on 40th day.

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* Written in Japanese

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吉田肉腫の移植に必要な細胞の数に関する研究
(1箇の細胞による腫瘍の移植)

石橋嘉久藏

(東北大学医学部病理学教室　指導　吉田富三教授)

吉田肉腫の腫瘍腹水を正常ダイコクネズミ腹膜腔液添加生理的食鹽水で稀釋し，その小滴中に含まれる細胞數を顯微鏡下に嚴密に數え，これをミクロマニプラトールにより，ピペット中に吸い取り，正常ダイコクネズミの腹膜腔内に注入する方法により，細胞數 11 箇，2 箇及び 1 箇の場合にも移植に成功した。

一方，同樣に腫瘍腹水を稀釋し，これを遠心した上清の小滴中に，細胞の全然ないことを一つ一つ確認したもののみを吸い取つて，正常ダイコクネズミの腹膜腔内に注入した試驗は，總て移植陰性と認むべき成績に終つた。

この際，無細胞の場合の稀釋は 4 倍～17 倍であるのに對し，細胞 1 箇の場合の如きは，腫瘍腹水が約 500 萬倍程に稀釋されている。卽ち稀釋の程度が低くとも無細胞の場合は植わらないが，極めて高度の稀釋をした場合でもその中に細胞が含まれていれば，それで植わると言う結果になつている。

又少數細胞移植で目立つことは，腫瘍の成長に要する時間の延長することである。腫瘍腹水の 0.01～0.02 cc, 細胞數にして 100 萬～1,000 萬箇を注入している日常の移植では，腫瘍細胞が腹膜腔内で純培養狀態に增殖する迄に 3～4 日を要するのであるが，細胞數が 3 萬箇になると，これが 7 日に延び，8,000 箇或は 2,000 箇になると十數日に延びる。しかし，20 箇或は 1 箇になつても，最早や大差はなく；大體 2 週間前後となつている。

Fig. 1 Micropipettes

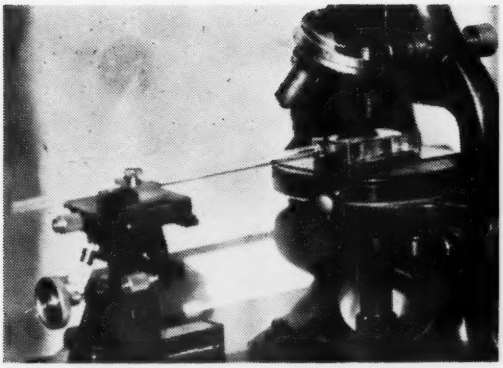

Fig. 2 Micromanipulator

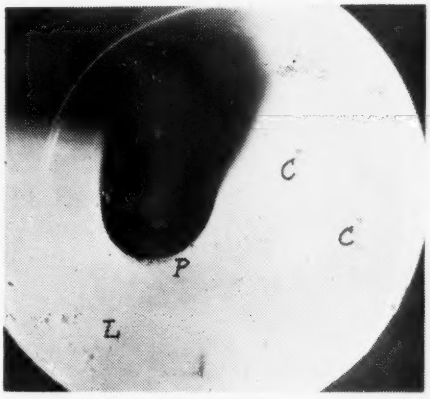

Fig. 3 At the moment of a droplet (containing 2 cells) being sucked into the micropipette.
C: Tumor cell.
P: Tip of the micropipette.
L: Outline of the droplet.

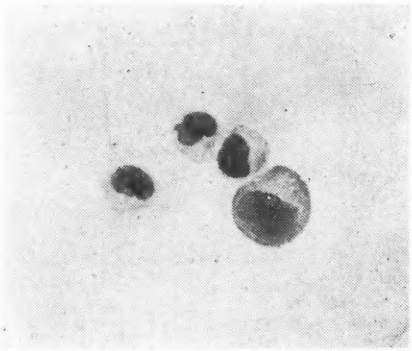

Fig. 4 Microscopic picture on the 9th day after the inoculation of 20 cells; right, 1 tumor cell; left, 3 normal monocytes.

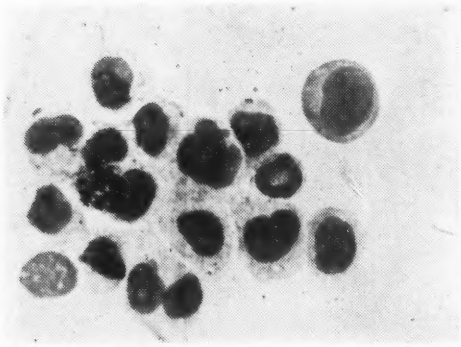

Fig. 5 On the 12th day after the inoculation of 20 cells; there is no transitional form between large monocytes and tumor cell.

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STUDY ON CARCINOSARCOMA OF STOMACH

(With Pl. III)

SHIRO KITAMURA

Pathological Department, Niigata Medical College
(Director: Prof. Akazaki)

INTRODUCTION

Carcinosarcoma of stomach is so rare, in comparison with that of other organs, that there have been only ten cases reported since Queckenstedt in 1904 made the first report on a case of the said disease. As I have recently had the opportunity of treating two cases with the characteristics of carcinosarcoma, I am going to report here, having made a re-examination of them together with the case already reported by Furukawa of our department and other previous reports in this line of study.

Report of Cases.

Case 1. 64 years old. Female.

Clinical diagnosis: Carcinoma of stomach. Both family and previous own history unknown.

Present illness: Since about Nov. 1947 the patient had had the sensation of fullness and of stagnation in the gastric region after meals, and she also vomited from time to time. In December she came to feel the presence of tumor at the upper left of the navel. Having grown thinner with more frequent vomiting since the spring of 1948 she visited Takeyama Hospital on August 2; X-ray photograph showed carcinoma of stomach. Total gastric resection was performed on Aug. 17 with a very satisfactory result, enabling her to leave the hospital on Sep. 12. Little is known about the case subsequent to this date.

Only the resected material was examined.

Macroscopic findings: The stomach excised covered from cardia to pylorus and was rather small as a whole. The tumor was located at the lesser curvature side covering from cardia to the gastric isthmus, and infiltrating in the front- and backwall of the stomach with the lesser curvature as the axis, causing marked thickening of the stomach wall. On the other hand, the tumor had grown extensively into the omentum minus also, assuming the appearance of sarcoma. Then, on opening the stomach along the greater curvature, we found the tumor projecting almost up to the middle of the front- and backwall, symmetrically with the stomach street as its axis. Its inner surface was swollen,

covered all over with protuberances of all sizes. Although it wss partially in the state of necrosis, the greater part was covered with normal mucous membrane.

Two portions in the local lymph-nodes were swollen to the size of little-finger-tip, the section was grayish white and completely turned to tumor.

Microscopic findings: The microscopic sections obtained from ten or more portions of the tumor show that the tumor cells have extensively infiltrated in the submucosa, muscularis and serosa, but the greater part of the mucosa shows no signs of malignancy; the figure is that of chronic catarrhal gastritis with a number of plasma cells, lymphocytes and Russell's bodies. Only in a portion the tumor, growing from the submucosa into the lumen of stomach, assumes an appearance as though it replaced the mucosa itself. Each section reveals nearly the same structure; polymorph cell sarcoma, medullary cancer, or some tendency of scirrhus in portions. Sarcomatous cells are mostly of round or spindle-shape which are found either separately or mixed together. Further, there are also some giant cells present among them, showing polymorphic figure with marked karyomitosis.

Whereas the spindle cells show almost uniform shape with elongated nucleus and abundant chromatin, the round cells vary in their size; some containing much chromatin and being slightly larger than lymphocytes, others containing comparatively large and clear nucleus and little cytoplasm and still others containing less chromatin and much cytoplasm.

However, these can be distinguished from the cancer cells which are to be mentioned later. Now, the above mentioned cells are either tied up with one another or isolated, each however closely associated with argyrophile fibers.

On the other hand, where the figure of medullary carcinoma is shown, alveolar structure is formed with delicate connective tissue, and such cells are mostly polygonal or round, containing much cytoplasm, with large and vesicular nucleus; signetring cell formation is also observed. Where the stomach is more or less scirrhous, the cancer parenchyma is consisted of a small number of cells, some round and others more or less spindle-shaped. In some portions the carcinomatous part and sarcomatous one can be clearly distinguished, in others the two are mixed, and in still others they have undergone very natural transition to each other.

Case 2. 40 years old. Female.

Clinical diagnosis: Tumor of stomach with subphrenic abscess.

Family history and case history; nothing abnormal.

Present illness: Since February 1946, the patient had complained of heart-burn, loss of appetite and general fatigue. Since August she had a feeling as if her stomach were squeezed. On Oct. 20, while taking a meal, she felt a

severe pain in the left side of the abdomen and vomited in the meantime.

Her doctor gave the diagnosis as peritonitis, notifying her of the presence of purulent mass in her left hypochondric region. X-ray photograph revealed the presence of tumor arising from the stomach with no abnormality in the cardia und pyloric region. On Jan. 7, 1947, by test laparotomy, subphrenic abscess was detected.

Three days after the operation the patient began to vomit something like coffee grounds and died on Feb. 2.

Autopsy report: chief findings are as follows:

The widely opened operation wound, approximately 7 cm. was seen along the external margin of M. rectus abdominis of the left hypochondric region, and it was found, by means of sounding, to be opened to the lumen of the stomach. Nothing peculiar was found in the lower region of the abdominal cavity.

In the epigastric region, the tumor was found to be infiltrating all over the gastric region with the stomach body as the center, thickening the stomach wall markedly and covering a part of inner surface with fungous protuberances. The said fistula opened to the transitional portion of the stomach body and the isthmus in gangrenous form, but the greater part was covered by normal mucous membrane.

The tumor growing outside of the stomach has affected a part of the left lobe of the liver, the tail of pancreas and the transverse colon, forming a single colossal lump. Although the left kidney, the left adrenal and the spleen are partly buried in the tumor, it has not invaded these organs. No metastasis is seen anywhere.

The findings besides the above mentioned are:

1. The submucous fibromyoma of soy-bean-size in the anterior wall of the uterus.

2. The fibroma of lentil-size in the left ovary. (The rest of the findings are omitted here.)

Microscopic findings: As was supposed by macroscopic observation, the tumor was of gastric origin, infiltrated into the muscularis in high density and extensively into the submucosa and serosa; whereas the mucous membrane, except the above mentioned gangrenous portion, was fairly intact. In some portions, however, plasma cells and Russell's bodies were seen quite clearly.

The tumor structure was the same everywhere, mostly consisting of round cell sarcoma. On the contrary, what was discerned as medullary cancer occupied only a small bit of portion. The round cells of the sarcoma being of varied sizes, some were of lymphoid form, others were round with much cytoplasm and clear nucleus, and still others were of the meso-type of the above two with

marked karyomitosis. Because of the insufficient mutual connection of these cells, most of them clustered in isolated groups, although there were some exceptional ones which keep better connection. Argyrophile fibers showed a close relation with these various cells. On the contrary, in the portion of medullary cancer, alveolar structure was formed by delicate connective tissue, and the cells were polygonal or round with large vesicular nucleus. Such medullary cancer has turned to sarcomatous figure in a most natural way. Not only in the parts of the medullary cancer, but also in the sarcoma the signetring cell formation was detected.

The cells which are apparently identical with the said carcinoma cells were scattered in the sarcoma. The tumors in the liver, the pancreastail and the transverse colon showed the same microscopic structure as above. In the liver, alveolar structure was discerned slightly more clearly, and here also the carcinoma had urnedt quite naturally into sarcoma.

Case 3. 46 years old. Female.

Since this case has already been reported on by Furukawa of our department (No. 5, 60th year, Niigata Medical Association Bulletin) I am going to make a rather brief statement here.

Clinical diagnosis: Carcinoma of stomach.

Present illness: Since June, 1945, the patient complained the loss of appetite and stomachache. A medical examination detected tumor in the gastric region. As the tumor gradually grew bigger, the patient had her stomach resected at Takeyama Hospital on Dec. 17. According to the clinical findings no metastasis of the tumor was detectable.

Macroscopic findings: The stomach tumor taken out was located on the side of the lesser curvature covering from cardia to pylorus and swelling up in tumorous fashion toward the lumen of stomach. Though a part of the inner surface showed ulcer formation, the greater part was covered with the characteristic mucous membrane reminding one of the sarcoma originated from the muscular layer of stomach.

Four of the lymphnodes adjacent to lesser curvature were found swollen from rice-grain to pigeon-egg size.

Microscopic findings: The majority of the tumor cells consisted of round cells, most of which had, with some few exceptions of lymphocyte-like cells, abundant cytoplasm with round and clear nucleus. Mixed with these were sometimes large round cells with vesicular nucleus.

Where such sarcomatous appearance was maintained, argyrophile fibers thickly projected among the cells. However, further investigation showed some portions where alveolar structure was formed by means of delicate connective

tissue, with rather marked binding of the cells with one another, suggestive of carcinoma.

Close to the mucous membrane atypical carcinomatous figure with adenoid structure was detected. Such carcinoma gradually turned to sarcoma; and signetring cell formation was detectable not only in carcinoma but also in sarcoma. The same is true with microscopic figure of lymph-node metastasis.

Comments

Although the above mentioned three cases slightly differ from one another in reference to microscopic structure, all show the tumor cells infiltrating thickly as well as extensively in the submucosa, muscularis and serosa; marked thickening of the stomach wall; partially growing toward the lumen of stomach; giving the impression as though the mucous membrane has been replaced by the tumor.

Furthermore, around the mucous membrane, which has turned tumorous, a marked figure of chronic catarrh is detected with a large number of plasma cells, Russell's bodies and lymphocytes. Though in the minute structure these three tumors also vary a great deal from one another, they all consist of round or spindle cell sarcoma on one hand, and medullary cancer (partially scirrhus or adenocarcinoma) on the other, the two components showing no parenchyma-stroma relation but naturally and gradually turning to each other.

If we assume that all the tumor cells in the sarcoma to be epithelial cells identical with the polymorphic or large round cancer cells, these tumors are sarcomatoid carcinomas (carcinoma sarcomatoides); but as the argyrophile fibers thickly projecting among these sarcoma-cells and presenting no alveolar structure, they are identified with what we commonly call sarcoma.

The cells identical with the above mentioned cancer cells which are found scattered among the sarcomas may be rightly taken as epithelial cells mixing in the sarcomatous structure. From such a view, the above mentioned are evidently cases of carcinosarcoma.

The problem with the second and the third cases is that no evidence can be given as to whether the signetring cells dispersed in the sarcoma have their origin in the epithelial cells scattered in the said sarcoma or those cells resembling sarcoma cells are epithelial by nature, in part participating in signetring cell formation; which renders the determination as carcinosarcoma difficult.

However, in the first case, no signetring cell formation is seen in the sarcoma, the mutual connection among the sarcoma cells being less sufficient than in the other two cases, and also the figure of spindle cell sarcoma being

prevalent, this case ought to be determined as carcinosarcoma.

On the other hand, it is a rather natural inference that the mutual connection of the sarcomatous cells often seen in the second and the third cases indicates more or less epithelial nature on the assumption of the embryonal entodermmesenchymal origin of this tumor.

Furthermore, it is practically impossible to determine epithelial or non epithelial nature simply from tumor cells themselves. The determination should be made on the basis of the general microscopic picture including parenchyma-stroma relation, the state of the nuclei, the relation between the argyrophile fibers and the sarcoma cell.

From this point of view, it is most appropriate to name the said three cases carcinosarcoma. As for metastasis, also, they all show approximately the same structure as primary tumor; the sarcoma and the carcinoma being mixed without any parenchyma-stroma relation or turning to each other most naturally and gradually; these are tumors with the same origin as to their ancester cells, belonging to Combination tumor after R. Meyer's classification.

Finally I offer the material for general observation based on the above three cases and those reported in literature. See also Table

1. Sex and age: Out of 12 cases of carcinosarcoma of stomach given in the table, the proportion between men and women is 6:6. As to the age, 4 in sixties, 3 in fifties, 3 in fourties and one thirty-eight and another twenty-nine. The average, being 50.4 years, agrees with the so-called cancer age.

2. Origin of development: 7 cases in the pyloric region, 2 in and around the stomach body, 3 in wide area with the lasser curvature as the center.

3. Macroscopic appearance: What is most noticeable morphologically is the marked thickening of the stomach wall. This is particularly marked in 9 cases out of 11 (One case is not enumerated, because Queckenstedt's original work is not at hand). The following is the classification by previous authors, according to the state of the stomach wall.

A-type: The outstanding characteristic is the general thickening of the stomach wall. (Lindemann, Saito, Konjetzny's 2nd case, Kawano)

B-type: Showing the development chiefly toward the outside of the stomach wall. (Kitamura's lst and 2nd cases)

C-type: Showing the development chiefly toward the inside of the stomach wall. There are two sub-types belonging to this, namely;

1) Those which show a number of semiglobular or fungous swellings in the lumen of stomach (Schuback, Furukawa).

2) Those showing isolated fungous or crater-shaped swelling in the lumen of stomach (Konjetzny's Ist case, Nakazawa-Kaneko, Gotting).

Thus these types generally show sarcomatous characteristics and three cases in A and B types showed perforation (Konjetzny's 2nd case) or gastric fistula formation (Lindemann, Kitamura's 2nd case)

4. Metastasis: Out of 12 cases 8 show metastasis; 5 the same structure as the primary lesion; 2 only carcinomatous structure; one unexamined.

5. Classification of carcinosarcama in reference to its formal genesis: As to the origin of carcinosarcoma, Götting, Herxheimer, Konjetzny, etc., commented on various possibilities, somewhat as follows:

1) Carcinoma and sarcoma being separately originated and a single tumor being made by mutual contact and mixture—the tumor corresponds to Collision tumor in R. Meyer's classification.

2) The carcinoma being primarily originated, the stimulation there turns the stroma to sarcoma; or the sarcoma being primarily originated, the epithelium enclosed in it turns to carcinoma.

3) Epithelial or non-epithelial cells developing, from the same cause, simultaneously or with a slight lag of time, both taking the course of malignacy.

2) and 3) correspond to Meyer's Composition tumor.

5) The cells originated in those which were left behind the normal development of embryonal period, i.e., in the ancester cells. This corresponds to R. Meyer's Combination tumor.

5) Carcinoma cells metaplastically turning to sarcomatous cells.

Suppose we classify, according to Meyer's classification, the carcinosarcomas of stomach hitherto reported, there are 3 cases of what the reporter himself named Collision tumor (Schuback, Nakazowa and Götting) and 2, which can be identified as the same (Kawano, Konjetzny's 2nd case), 3 cases of Composition tumor (Lindemann, Konjetzny's 1st cases, Saito) and 4 called Combination tumor (Queckenstedt, Furukawa, Kitamura's 1st and 2nd cases). However, as to Queckenstedt's case, whereas he himself traces the origin back to the embryonal entoderm-mesenchymal germ, Schuback and Götting classify this case as Collision tumor and Composition tumor, respectively. Also, as to Konjetzny's 2nd case, whereas the reporter himself states "It is most probable that the stroma of the cancer turned to sarcoma in the secondary transition", Götting and Nakazawa definitely clossify this case as Collision tumor. In fact, the determination of tumors as to whether they are Collision or Combination tumor simply from the microscopic picture is not easy, and also it can easily be supposed that, in case the components of Collision tumor develop very close with each other, the tumor shows the same microscopic picture as Composition tumor or Combination tumor. As this cause much inconvenience in classification, I would suggest, aside from the determination of the formal genesis of

carcinosarcoma the following classification from purely morphological stand point;

1st type: those tumors in which pure carcinoma and pure sarcoma occupy the greater part separately, the two showing mutual contact and mixture only in a minor part. This kind is consisted of two sub- types: that in which carcinoma and sarcoma have their mutual contact outside (chiefly corresponding to R. Meyer's Collision tumor) and that in which the one surrounds the other.

2nd type: The carcinoma and the sarcoma keep parenchyma- stroma relation. Here and there pure carcinoma and pure sarcoma occupy different parts separately, but in greater parts showing parenchyma-stroma relation, This can be explained either as the two components of Collision tumor developed very close to each other, or as what is called Composition tumor and Combination tumor. This type also includes, as its atypical form, those tumors which are partially occupied by pure carcinoma (or pure sarcoma), the major part consisting of carcinoma and sarcoma in parenchyma-stroma relation. Most probably this may be Composition tumor.

3rd type: Pure carcinoma and pure sarcoma being present in some parts separately, the two kinds quite naturally change to each other and well mixed together without showing parenchyma-stroma relation. This type is seen in R. Meyer's Combination tumor exclusively.

Summary

1. Report has been made on two new cases of carcinosarcoma of stomach, also recording what Furukawa of our department has previously reported on.

2. The above three cases all show the tumors which chiefly infiltrated the deep layer of stomach wall and partly protruded in the lumen of stomach, giving the appearance of the tumor partially replacing the mucous membrane. From histological view, they are composed of round or spindle-shaped cell sarcoma and medullary cancer (in part scirrhus or adenocarcinoma); the two components, in some portions, can be clearly distinguished from each other, but in most cases naturally transform to each other or well mixed up, showing no parenchyma-stroma relation.

3. All these three belong to R. Meyea's Combination tumor, originated in entoderm-mesenchymal germ left behind in the course of normal embryonic growth. However, in the second and third cases, signetring cell formation is seen in a part of the sarcoma, too, giving rise to the question whether this be originated in the epithelial cells scattering mixedly in the sarcoma or those sarcomatous cells be also really epithelial in nature, participating in signet-

ring cell formation. Thus it is impossible for us to determine this as carcinosarcoma. The first case, nevertheless, should be determined as carcinosarcoma, since no such signetring cell formation is seen in the sarcoma.

4. There have been only twelve cases of carcinosarcoma reported, including mine, up to the present. The sexual variance is 6 : 6 ; the average age 50.4. As to the origin of development, pyloric region comes first, there being 7 cases of it. Metastasis is seen in 8 cases out of 12, among which 5 are of the same structure as primary tumor, two carcinoma alone, one unexamined.

5. Macroscopic classification: The specially noticeable macroscopic characteristic is the marked thickening of the stomach wall. I have classified carcinosarcomas into 3 types according to the condition of the stomach wall.

A-type: Those which show chiefly the general thickening of the stomach wall.

B-type: Those which develop toward the outside of the stomach wall.

C-type: Those which develop toward the inside of the stomach wall. Dividing this up into two sub-types we get:

1) Those which show a number of semi-globular or fungous swellings in the stomach cavity.

2) Those showing isolated fungous or crater-shaped swelling in the stomach cavity.

6. Although it is both ideal and necessary to classify carcinosarcomas by formal genesis, it is extremely difficult as well as inconvenient to determine it by means of microscopic picture. I suggest a method of classification on the purely morphological stand point, that is:

1st type: Those in which pure carcinoma and pure sarcoma occupy the greater part separately, the two showing mutual contact and mixture only in a minor part. This kind is consisted of two sub- types: that in which carcinoma and sarcoma have their mutual contact outside, and that in which one surrounds the other.

2nd type: The carcinoma and the sarcoma keep parenchyma-stroma relation. Here and there pure carcinoma and pure sarcoma occupy different parts separately, mostly showing parenchyma-stroma relation.

3rd type: Pure carcinoma and pure sarcoma being present in a minor part separatery, the two quite naturally turning to each other, showing no parenchyma-stroma relation.

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Table

Name of reporter	Age	Sex	Orign of development	Metastasis	Macroscopic classification	Histological classification
Queckenstedt (1904)	54	Female	Pyloric region	(—)	Uncertain	Uncertain
Lindemann (1908)	46	Male	Pyloric region	Carcinosarcoma	A-Type	II-Type
Saito (1916)	29	Femals	Whole stomach with lesser culvature as the center	Carcinosarcoma	A-Type	II-Type
Konjetzny (1921)	60	Male	Pyloric region	Carcinosarcoma	C-Type$_2$	II-Type
Konjetzny (1921)	60	Female	Pyloric region	(—)	A-Type	I-Type
Götting (1931)	38	Male	Pyloric region	Unexamined	C-Type$_2$	I-Type
Schubak (1931)	52	Male	1/3 upperpart of stomach	Carcinoma	C-Type$_1$	I-Type
Kawano (1933)	55	Male	Pyloric region	Carcinoma	A-Type	I-Type
Nakasawa (1937)	61	Male	Pyloric region	(—)	C-Type$_2$	I-Type
Furukawa (1946)	46	Female	From cardia to pylorus with the lesser culvature as the center	Carcinosarcoma	C-Type$_1$	III-Type
Kitamura (1949)	64	Female	From cardia to Isthmus with the lesser culvature as the center	Carcinosarcoma	B-Type	III-Type
Kitamura (1949)	40	Female	Whole stomach region with the stomach body as the center	(—)	B-Type	III-Type

Cases 12	Average Age= 50.4	Male : 6 Female : 6	Pyloric regin : 7 Stomach body : 2 Greater part of stomach with the lesser culvature as the center : 3		A-Type : 4 B-Type : 2 C-Type 1 : 2 C-Type 2 : 3 Uncertain : 1	I-Type : 5 II-Type: 3 III-Type : 3 Uncer tain : 1

Plate III

Case I.

1. Signetring cell formation in medullary cancer.
2. Polymorph cell sarcoma.
3. Argyrophile fibers in sarcomatous portion.

Case II.

1. Medullary cancer.
2. Signetring cell formation in sarcomatous portion.
3. Argyrophile fibers in sarcomatous portion.

Case III.

1. Adenocarcinoma.
2. Signetring cell formation in sarcomatous portion.
3. Argyrophile fibers in sarcomatous portion.

要　旨

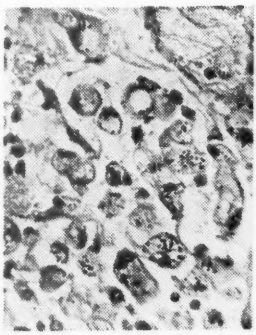
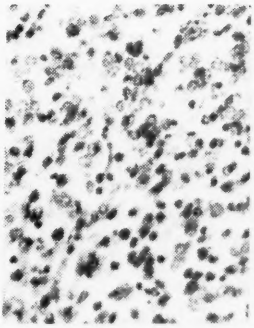
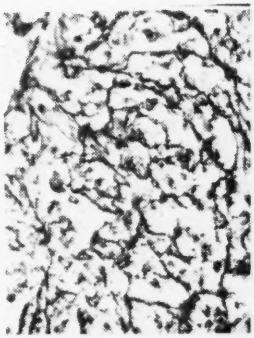
胃の癌肉腫に就いて

北村四郎（新潟医科大学病理学教室 指導 赤崎兼義）

胃の癌肉腫は他の臓器のそれに比し稀で，1904 年 Queckenstedt が初めて報告してから現在迄僅かに 10 例を數へるにすぎない。著者は最近癌肉腫と思われる 3 例（内 1 例は既に當教室の古川が報告）を經驗したので之迄の諸家の報告と合せ檢討し，ここに報告する。著者の例は何れも一方には圓形乃至紡錘形細胞肉腫と他方には髄様癌の像が認められ，兩者は實質と間質の關係を示さず，自然に何時の間にか移行している。此の點よりすれば 3 例共に一つの源基細胞に由來する腫瘍と考えられ，R. Meyer の分類に從へば Combinationstumor に屬する。唯 3 例の内 2 例には肉腫内にも印環細胞形成が認められるので，或いは肉腫様構造を示す癌腫と考えられぬ事もない。然し此の肉腫内に於ても嗜銀性纖維は腫瘍細胞と密接な關係を有し，吾々が平常肉腫と呼んでいる像に完全に一致しているので，3 例共に癌肉腫と判斷するのが至當と考える。尚著者は之迄に報告された癌肉腫の所見を基礎として肉眼的には胃壁の肥厚の狀態により之を瀰蔓性肥厚をなすものと，胃壁の内外に夫々發育する型に大別し，組織學的には純形態學的な見地から分類を試みた。

Case I

1 2 3

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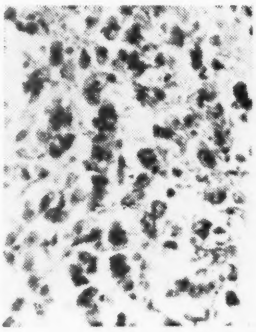
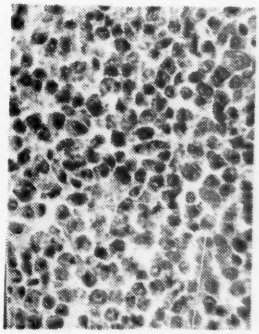
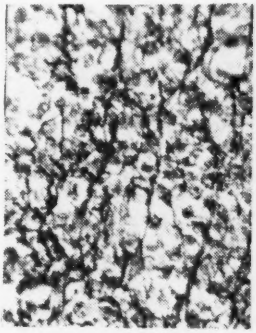

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Case III

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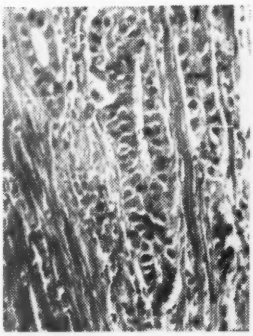
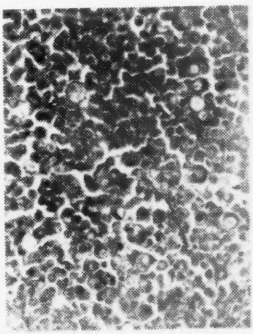



[GANN, Vol. 41; March, 1950]

STUDIES ON THE EXPERIMENTAL INTERRUPTION OF THE FERMENT SYSTEM OF GLUCOSE METABOLISM IN THE SARCOMA PRODUCING TISSUES. IST REPORT.

(With Pl. IV)

SHIGEYASU AMANO, YOSHITADA TAKINO and YUSAKU TAGASHIRA

Department of Pathology, Medical Faculty, Kyoto University

PREFACE

Nishiyama (1938) succeeded in the experiment to produce sarcomas through a long continued application of concentrated glucose solution in subcutaneous tissues of the rats. Our interest in this experiment depends on the fact that this sarcoma provocator, glucose, prevails physiologically in organisms and also on the knowledge that the only characteristic metabolism of the tumor may be attributed to the modus of consuming this glucose. We could succeed in the same production of such a sarcoma more effectively through the injection of glycogen (Amano-Ito, 1943, Ito, 1944) than of glucose. Takizawa (1938, 39) confirmed Nishiyama's experiment and, furthermore, produced sarcoma through the injection of lactose or fructose.

Although the use of various cancerogenic substances is reported heretofore, there is no proper explanation about determinative biochemical process of cancerogenesis in tissue cells. In our case, we tried to get some knowledge concerning this biochemical process of cancerogenesis. In the previous papers dealing with glycogen injection we assumed a physico-chemical condition producing the nuclear mutation; here we will make again an appraisal on the same problem from a new point of view, concerning the glucose-matabolizing system in the cell protoplasma.

It is a well known fact that G. Embden and M. Zimmermann (1924) through the addition of fluoride or oxalate to minced muscles or their juice observed a remarkable decrease of inorganic phosphate, accompanying the accumulation of hexose-diphosphate; this fact was acknowledged, thereafter, by Beatti and Milroy (1925) and Euler et al. (1925). Meyerhof (1926) explained that this action of fluoride or oxalate is not due to their ability to accelerate the ester formation, but to their nature to inhibit the decomposition of the ester there. He stated that the decomposition of the polysaccharides of frog muscle (extracted with KCl solution) may be inhibited to 80% by the addition of N/100 NaF, following the the inhibition of the ester formation according to the concentration of the fluoride. Such a function of fluoride was summarized by F. Lipmann (1928, 29),

K. Dohmann (1933) and Meyerhof (1933) as " A selective inhibition of the formation of pyruvic acid from glycerin-2-phosphoric acid."

According to E. Lundgaard (1930), moreover, the injections of monoiodoacetic acid intravenous in rabbits or in the lymphsac of frogs, produce a selective disturbance of the striated muscles, in which the formation of lactic acid was strongly depressed (N/5000 of the chemicals inhibited completely the sugar decomposition). And Meyerhof and W. Kissling (1933), afterward, cleared the fact by saying that " the formation of pyruvic acid and glycerophosphoric acid is inhibited by monoiodoacetic acid ". There is no doubt that those two inhibitory substances stand in intimate relation to the enzyme system of the sugar decomposition, because those stoppages of the sugar metabolism do not recover by the addition of co-enzyme.

It is our hypothetical assumption that such substance that attack selectively some steps of the sugar metabolism in vitro, may play the same role in vivo in those proliferating tissues burdened with concentrated glucose, that is to cut the enzyme system of those tissues. In other words, the formation of glucose-sarcoma may be influenced in some way through sodium fluoride or monoiodoacetic acid, according to their modus of selective inhibition of the process of glucose decomposition. From this point of view, our first experiment was planed in the following way.

Material

As cancerogenic substance purified glucose (Takeda Co.) and as an accelerating factor o-amidoazotoluene in olive oil were used. Inhibititors used were sodium fluoride (Daikokuya) and monoiodoacetic acid (Toakagakukogyo). Experimental animals were over one hundred white rats for the preliminary and main experiments.

The Dosages of Sodium Fluoride and Monoiodoacetic Acid.

70-110 gr white rats were injected daily subcutaneously with 0.4 g/dl sodium fluoride or 1.0 g/dl monoiodoacetic acid. The changes of the body weight and the mortality of the animals were observed at various periods. The following two experiments indicate the opimal dosage of the chemicals. (See Tables I and II)

Table I. Dosage determination of sodium fluoride (0.4 g/dl)

No.	Body weight Beginning	End	Number of inject.	Result	Dose cc per day	Dose per 100 g body weight (mg/per day)
1	72	59	3	Died	1.50	8.33
2	72	60	3		1.50	8.33
3	72	61	3		1.50	8.33

4	96	85	3		1.50	6.03
5	100	83	3		1.25	5.00
6	65	62	1		0.75	4.61
7	65	61	1		0.75	4.61
8	85	80	5		0.75	3.55
9	75	50	11		0.50	2.66
10	83	70	19		0.50	2.44
11	75	76	21	Survived	0.45	2.40
12	87	92	21		0.45	2.07

Table II. Dosage determination of monoiodoacetic acid (1 g/dl)

No.	Body weight Beginning	End	Number of inject.	Result	Dose cc per day	Dose per 100 g body weighf (mg/per day)
1	57	57	1	Died	0.35	6.15
2	112	108	2		0.50	4.46
3	87	84	3		0.35	4.02
4	75	72	21	Survived	0.30	4.00
5	118	90	21		0.45	3.98
6	110	112	21		0.30	2.72
7	74	36	20	Died	0.25	2.65
8	101	74	4	Died by accident	0.25	2.47

These results show that white rats with the weight of 100g, injected for 3 weeks daily with 2.4 mg sodium fluoride or with 2.5 mg monoiodoacetic acid were in a somewhat sound condition.

Taking these facts into consideration we used both chemicals in the following main experiments in the concentration of 0.4 g/dl, injecting 0.5cc subcutaneously every other day.

Beside those given in the above, we made many other experiments for the same purpose. The animals that died as well as those that survived were examined histologically. Our dose of sodium fluoride was about 2 mg daily, which is much smaller dose than that used by Nishi (1943), who had made an experiment to study the toxicity of this chemical. According to Nishi, 0.5-0.7 gr of sodium fluoride given daily is ample to bring forth dental changes such as mottled teeth and overgrowth of teeth in addition to osteosclerotic changes, while the general condition is not remarkably disturbed. In our dose, the animals showed distinctly mottled teeth after one month, and in case of further application a malnutrition of hair was observed, but no other disturbance. As for the monoiodoacetic acid group, there was no remarkable change of the general conditions. These results may be seen in the body weight curves of

these animals in the main experiments (Chart 1).

The Main Experiment

Three groups of rats were prepared for this experiment.

1st group: 9 white rats were injected subcutaneously with 1 cc of 20% glucose solution and 0.5 cc of 0.4 g/dl sodium fluoride solution every other day.

2nd group: 9 white rats were injected subcutaneously with 1 cc of 20 % glucose solution and 0.5 cc of 0.4 g/dl monoiodoacetic acid solution every other day.

3rd group: 8 white rats were injected subcutaneously with 1 cc of 20% glucose solution and 0.5 cc of saline solutuion every other day.

As far as possible the same area on the back of the animals was used for the locus of the injection.

Moreover, the injection of 0.3 cc of 5 % o-amidoazotoluene in olive oil was continued weekly during the first experimental month and thereafter every other week in the same site.

Results

1st group (sodium fluoride group): of 9 rats of this group 7 survived more than 200 days (one of them producing sarcoma) and 4 lived more than 365 days (2 of them producing sarcoma). (See Table III.).

Table III. Sodium fluoride group

No.	Sex	Exp. days	Inject. No.	Body weight (g) Beginning	End	Sarcoma
1	♂	329	155	140	300	Spindle cell sarcoma (56×42×35)mm
2	♂	458	180	150	230	
3	♂	214	100	150	230	
4	♂	384	180	180	315	
5	♀	382	180	155	185	Spindle cell sarcoma (28×17×12)mm
6	♀	385	180	150	180	Polymor. cell sarcoma (34×33×24)mm
7	♂	319	150	200	255	

In most of the rats that received 1 or 2 injections, the locus was resolved completely, but after several injections there remained in the subcutaneum some movable indurations, which increased in size following further injections. But these indurations were not so intense as those of the 2nd group; hyperplasia

of the epidermis and adhesion of this induration to the basis were not remarkable.

2nd group (monoiodoacetic acid group): Of 9 rats of this group 6 survived longer than 200 days, among which 4 rats produced sarcoma before 300 days. Another rat formed polymorph cell sarcoma at the end of 198 experimental days injected 95 times). The sarcoma growth of this group was in all rats very active and no animal survived till the end of this experiment (Table IV).

Table IV. Monoiodoacetic acid group

No.	Sex	Exp. days	Inject. No.	Body weight Beginning	Eed	Sarcoma
1	♂	296	137	170	325	Polymor. cell sarcoma (65×55×45)mm
2	♂	314	139	150	295	Spindle cell sarcoma (65×44×34)mm
3	♂	314	139	185	295	Polymor. cell sarcoma (65×64×45)mm
4	♂	313	100	170	205	Polymor. cell sarcoma
5	♀	285	107	150	250	Polymor. cell sarcoma (74×50×45)mm
6	♀	255	98	145	270	Polymor. cell sarcoma (65×56×43)mm
7	♂	198	99	190	240	

In this group the local change was generally very intense. The first injection evoked an edematous infiltration of the subcutaneum, which alluded hyperplasia of the epidermis and the adhesion with the tissue below. After 7 or 8 injections the locus become very hard, so that the newly injected solution flows sometimes back from the hole of injection. Furthermore, after 40-60 experimental days, there appeared a somewhat linear folding of the epidermis. After 3 or 4 months the resorption of the injected solution became more and more difficult. Therefore, the solution remained in form of an abscess in these connective tissues, with bleeding tendency. The continued injection into such subcutaneum produced new harder nodules at the place of such infiltration, which grew rapidly to tumors.

3rd group (NaCl solution group): Of 8 rate in this group 7 survived longer than 200 days, of which 5 rats remained more than 365 days. 3 of those 5 rats produced sarcoma (Table V).

Table V. NaCl group.

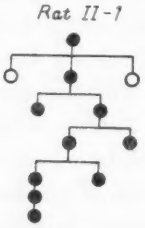
No.	Sex	Exp. days	Inject. No.	Body weight Beginning	End	Sarcoma
1	♀	324	180	130	195	

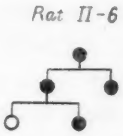
2	♂	382	180	195	290	Spindle cell sarcoma (40×30×17)mm
3	♀	308	150	160	145	
4	♀	385	180	125	160	Polymor. cell sarcoma (35×28×23)mm
5	♀	385	180	150	180	Polymor. cell sarcoma (25×22×18)mm
6	♀	368	180	130	150	
7	♀	277	130	175	200	

The local change in this group was generally very slight. Even after continuous injections we observed only a small infiltration consisting of groups of miliary nodules. Histologically the islets of small necrosis were surrounded with exudate cells (including plasma cells) and connective tissues accompanying capillaries were recognized. The hyperplasia of the epidermis was also very slight in this group.

In reference to the sarcoma produced in the 1st and 3rd groups (with the exception of rat No. 1 of the 1st group), it was not easy to determine the malignancy of the tumor macroscopically. The tumor tissues and their tendency of growth was also weak.

With these primary sarcomas homoiotransplantation of the tumor tissues from rat No. 1 and No. 6 of the 2nd group were tried and with success. The strain of the former remains till now through continuous transplantation.





In this main experiment, monoiodoacetic acid and sodium fluoride were used in their maximal dosage. But their general influence may be seen in the following body weight curves (see Chart 1).

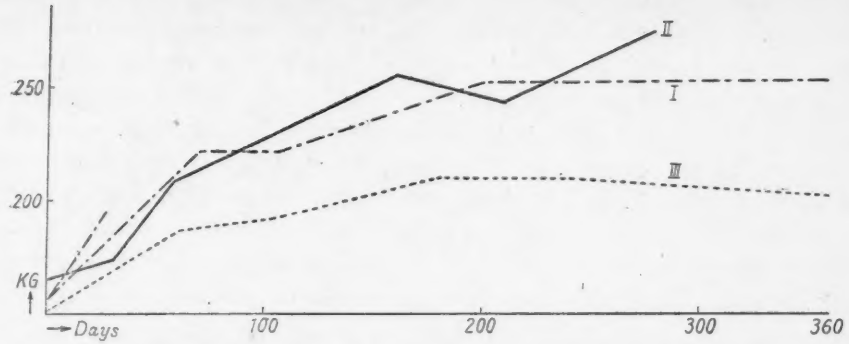


Chart 1. Average body weight of the animals (mean value) in each group

From this chart we learn that the influence of the chemicals on the nutrition of the animals is not so remarkable, compared with the influence on the modus of the sarcoma formation.

Discussion

On account of this main experiment, one can conclude that the formation of sarcoma by glucose injections is much facilitated by the additional injections of monoiodoacetic acid, while uninfluenced by the additional use of sodium fluoride. Concerning our point of view as to the suger metabolism, the results mean that glucose plays its cancerogenic role vividly in the stage before its breakdown to triose. But in such a stage of sugar decomposition which is easily blocked by sodium fluoride, neither acceleration nor inhibition of the cancerogenicity of glucose was observed.

In the previous paper we tried to give an explanation in reference to the sarcoma producing potency of glycogen (Amano-Ito, 1943), from the physico-chemical view point that the sugar acted as a condition to cause alternate dehydration and successive hydration to such cells in nuclear division of the connective tissues, and excite the cellular mutation, to sarcoma cells.

Our present results tell us that the sugar, in a stage before its breakdown to triose (the form of which is here out of question) is active to produce sarcoma, the fact which does not contradict our physico-chemical theory.

Moreover, hand in hand with this experiment, we measured in vitro the inhibitory influence of monoiodoacetic acid or sodium fluoride on the sugar breakdown by tissue cells (see the next report concerning the measuring of glucose and lactic acid in such a condition).

W. Wilbrandt and L. Laszt (1933) reported that monoiodoacetic acid inhibits

the resorption of sugar from the intestine, more intensely, by subcutaneous injection than by peroral use. Considering this fact and referring to the experiment of Lundgaard, the monoiodoacetic acid injected subcutaneously, accompanied with glucose, may disturb the resorption as well as the decomposition of the latter, producing an overflow of sugar in the state beforé it is decomposed to triose. In fact, quantitative results that the addition of monoiodoacetic acid to the mixture of glucose and tissue fluid, excites an inhibition of the decrease of the reducing substance in that mixture. Therefore, it may be no overstimation to say that by this experiment we proved the fact that the injected glucose is cancerogenic when it acts before being reduced to triose.

Summary

1. The influence of monoiodoacetic acid and sodium fluoride on the experimental sarcoma production by way of subcutaneous injection of concentrated glucose solution was tested. Monoiodoacetic acid accelerated the sarcoma production, shortening the period and increasing percentage of the tumor formation, while sodium fluoride had no such effects.

2. From our assumption that the monoiodoacetic acid and sodium fluoride are acting to interrupt the ferment system of sugar metabolism in a certain stage, it may well be concluded on account of our results that the sarcoma producing hexose is only effective, when it acts in the stage before its breakdown to triose.

3. The dosage of monoiodoacetic acid and sodium fluoride here used did not effect any disturbance to the general nutrition of the experimental animals.

4. Nishiyama, who succeeded for the first time in the sarcoma production with concentrated glucose solution, used at the same time o-amidoazotoluene to yield good results. So we also used it as an olive oil solution in the same sense, but by way of subcutaneous injection to know accurate amount absorbed in the body, instead of the peroral application as Nishiyama.

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EXPLANATION OF PLATE IV

Fig. 1 : Rat No. 1, 2nd group, 298 days.
Fig. 2 : Rat No. 2, 2nd group, 298 days.
Fig. 3 : Rat No. 3, 2nd group, 298 days.
Fig. 4 : Rat No. 4, 2nd group, 210 days.

抄　　錄

發癌組織における糖代謝酵素系の切斷に關する實驗的研究　第1報*

天野重安・瀧野義忠・田頭勇作
(京大医学部病理学教室)

肉腫が濃厚葡萄糖の注射で發生せしめうるという西山氏の新知見は，この物質が生體に生理的に存在するだけに我々の關心を強くひく。我々は葡萄糖に代えて糖原を用いても同樣に肉腫の發生することを認めた(天野・伊東)。さて，今日腫瘍新陳代謝の特性と目せられているものは糖代謝形式である。その特殊の糖代謝形式と前述の强度の糖の負荷との間には必ずや重要な腫瘍發生の要約が陰されていることと考えられる。

* 本報の內容は昭和24年4月3日日本癌学会に於て報告した。

そこで我々はこの糖負荷に際する，糖代謝の何の部分が發癌性に作用するのであるかを窺知するため，糖代謝酵素系の切斷を計畫した。それは一沃度醋酸乃至は弗化曹達の使用によってこの糖分解をトリオーゼ以前の狀態に止めるとか，トリオーゼの段階に止める様にして，その場合に肉腫發生が如何に影響されるかを差當って調べることである。

この實驗には二種の豫備階梯が必要で，1. 長期間の一沃度醋酸乃至弗化曹達注射を行うに適した最高耐量を決定すること，及び，2. この耐量が同時に注射された葡萄糖の分解に明かに影響しうることを化學的定量的に確認しおくことである。過去數年の準備實驗によつてこれらの條件が滿足せられることが判明したので次の如き本實驗を試みた。

I 群 20% 葡萄糖 1cc. 隔日注射+0.4g/dl 弗化曹達 0.5cc 同時注射

II 群 20% 葡萄糖 1cc. 隔日注射+0.4g/dl 一沃度醋酸 0.5cc 同時注射

III 群 20% 葡萄糖 1cc. 隔日注射+0.8% 食鹽水 0 5cc 同時注射

なお各群の動物にはこのほかに 5% 0-Amidoazotoluol 0.3cc を週 1 回（第 2 ヶ月以後は 2 週間に 1 回）上記注射部位に注射した。

これらの動物は 200 日以上生存せるもの各群に 7 匹ずつあり，これらの動物中肉腫の發生しきたつたのは第 II 群（一沃度醋酸群）で，300 日までの經過中において殆ど全例に於て肉腫が陽性であり，これらから 2 移植肉腫株系を作りえた。第 1 群（弗化曹達群）及び第 III 群（對照群）からは同期間において肉腫の發生例は第 1 群に 1 例あり，第 III 群にはない，但し 1 年以後に於て此等の群からは少數の肉腫を發生した。（表參照）

処　　置	200 日以上生存（肉腫動物數）		365 日以上生存（肉腫動物數）
第 I 群 （弗化曹達群）	7 例	（1 例）	4 例　（2 例）
第 II 群 （一沃度醋酸群）	7 例 （1 例早期死亡）	6 例 （300 日迄に全例肉腫発生）	な　し
第 III 群 （NaCl・対照群）	7 例	（な　し）	5 例　（3 例）

この實驗で用いた葡萄糖及び 0-Amidoazotoluol 量は西山氏の場合に比して遙かに少量であるから，第 I 群及び第 III 群の肉腫發生が低率なことは必ずしも異とするに足りない。これに比すれば第 II 群，卽，一沃度醋酸の肉腫發生を促す性質は注目に値するものがある。このことは葡萄糖がトリオーゼに分解される以前の狀態において作用した場合によく發癌性を發揮することを物語るものであろう。なお弗化曹達のこの程度の注射量では 1 ヶ月後には特有の齒牙變化を呈するが，それ以上の中毒性變化を呈していない。

Fig 1

Fig 2

Fig 3

Fig 4

[GANN, Vol. 41; March, 1950]

GROWTH INHIBITING EXPERIMENTS ON YOSHIDA SARCOMA

MOTOHIKO TOBIOKA and KAZUO UEOKA

Laboratory of Environmental Medicine, Nagoya University
(Seizo Katsunuma)

INTRODUCTION

The series of experiments reported in this paper was started as attempts at the cultivation in vitro of Yoshida sarcoma. An incidental finding in the course of these attempts that while spleen extracts from normal rats promoted the growth of the sarcoma culture, those from the sarcoma bearing rats were less effective, suggested the possibility of immunity developing against the sarcoma. The growth promoting effect of homologous spleen was already found by A. Fischer (1) in the case of Rous chicken sarcoma, although, according to Brüda (2) tumor tissue grows better in sera of splenectomized animals than in those of intact ones. We conducted various experiments and were able to prove that undoubted immunity develops against second implantations of the sarcoma.

In connection with the immunity studies, we also investigated the effect of nitrogen mustard, certain hormones, placenta preparation, etc. As to nitrogen mustard, Gilman (3) and Jacobson (4) previously reported favorable results on lymphosarcomatosis and Hodgkin's disease. Concerning hormones, Huggins' (5) work on the effect of castration on prostatic cancer, and Fergusson's on that of female sex hormone indicated markedly favorable influence. Halberstaedter (7) on the effect of ovariectomy, Fels (8), Loesser (9), Adair and Herrmann (10) on hormone therapy, and Haddow, Watkinson and Paterson (11), Nathanson (12) on the effect of estrogens on various mammary cancer metastases, all reported favorable results. Our experiments with nitrogen mustard showed a rapid disappearance of sarcoma cells in ascites, but the effect was temporary and generally animals died relatively early on account of the secondary action of the drug (13).

Finally, paralleling the immunity experiments, we made cytological studies on the cells present in ascites, comparing them with those appearing in the case of the transplantation in normal rats, and examining supra vital findings, phagocytic activities, locomotive abilities, etc.

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Tissue Culture

Yoshida ascites sarcoma was cultured in the medium in which rat plasma, chicken plasma, chick embryonal extract, organ extract and ascites of the rat were mixed, and the influence of the extracts of various organs of the rats and ascites on the development of the tumor was tested by the rate of growth. The gist of the experiment of the tissue culture, and of the transplantation test in various organs was published in the Nagoya Medical Journal, 1947 and in my original work in the Annual Report of laboratory of Environmental Medicine of Nagoya University, 1948-49 combined volume. It was stated in this work that the spleen extract slightly promoted the growth of the sarcoma tissue, that the ascites of the normal rat especially promoted it in high degrees, and that the growth was suppressed by the spleen extract from immunized rats.

The various coelon liquids and the extracts of various organs promoted or inhibited the growth of Yoshida sarcoma tissue culture.

When the ascites of rats was added to the culture medium besides chick plasma and chick embryonal extract, the development of the cultured Yoshida sarcoma tissue was most highly promoted, the addition of the spleen extract produced comparatively less effect and brain, testis, or liver extract showed no promoting effect. The spleen extract of the tumor transplanted rats inhibited the growth of the tumor tissue compared with the spleen extract of normal rats, and the tissue developed more slowly than the jointly cultured chick embryonal heart.

Transplantation after Various Previous Treatments

After this tumor was transplanted in the subcutaneous tissue, it was removed by operation when the growth of the tumor was rapid and therefore the rats were affected, and then it was transplanted again in the abdominal cavity of those rats. It was observed that the transplantation of the tumor was completely inhibited by the immunity which has been gained by the first transplantation.

When these tumor cells were transplanted to the rats which had been given the previous treatment, the tumor cells disappeared at least in several days and the retransplantantion was negative. Since the retransplantation was negative on account of the first transplantation it was proved that the immunity was obtained by the above-mentioned method. The definite growth, and the removal, of the first tumor proved that these rats ware not naturally immune.

Chemical Agents

Various quantities of Nitrogen mustard (methyl-bis-β-chloroethylamine and tris-β-chloroethylamine) were injected in the abdomens of Yoshida sarcoma

transplanted rats, and the effective doses per 100 g of body weight were determined. The influences upon the development of this tumor of benzol, typhoid and paratyphoid mixed vaccine (which also caused the leucopenia), placental medical compound (Thmenine) and estron medical compound (500 universal units, water soluble Ovahormon) were examined. The tumor cells were not destroyed by methyl-bis-β-chloroethyl-amine but these were perfectly destroyed by tris-β-chloroethyl-amine. Tris-β-chloroethylamine was added to 10% glucose solution, 0.5 cc. of which corresponds to 1-0.1 mg per 100 g of the rat body weight. These medicines were injected into the abdomen of rats within 1-3 days after sarcoma cells were transplanted also into their abdomens.

Tumor cells soon disappeared in the ascites during 1-2 days after the injection, but the majority of the rats died. One rat, which had been injected with 2.5 mg per 100 g of body weight lived 21 days and died of quite another cause. Less than 2.5 mg per 100 g of body weight must be used in order to destroy the tumor cells and yet to exempt rats from death.

Benzol or typhoid and paratyphoid mixed vaccine did not prove effective. In one of the 10 rats to which we applied placental medical compound the tumor cells disappeared. In 2 of the 7 rats to which the sarcoma was transplanted and estron medical compound was given, the tumor tissue disappeared. For one of these two cured rats 8.4 cc of estron medical compound was applied for 12 days, but the rest of the seven rats were not influenced by this hormone.

Table 1

Results of the growth inhibiting experiments on Yoshida-sarcoma.

Method of experiment	Conditions of the experiment					Effect on growth
Culture	Cultured in the medium to which the spleen extract of the sarcoma rat was added					Inhibited
	Cultured in the medium to which the spleen extract of the normal rat was added					Promoted slightly
	Cultured in the medium to which the ascites of the normal rat was added					Promoted
Previous treatment	Method of the transplantation	Tuumor was nipped off	Days after transplantation	Transplatation for the	Days after transpl.	Days of the effect
	Sarcoma ascites ↓ Subcutaneous tissue		Within 1 week (6 days)		21	3
			Within 2 weeks (10 days)		67	1
			Within 3 weeks (17 days)		52	6

		Within 3 weeks (24 days)	second time	31	3-4
	Tumor tissue ↓ Subcutaneous tissue	Within 5 weeks (29 days)		35	3
	Sarcoma ascites ↓ Testis	2 days (left) 6 days (right)		17	2
	Sarcoma ascites ↓ Testis			247	2
	Sarcoma ascites ↓ Frontal eye chamber			51	2
Chemicals	Sarcoma ascites ↓ Abdomen	Nitrogen mustard (tris-β-chloroethyl-amine) (methyl-bis-β-chloro-ethylamine) Typhoid and Paratyphoid mixed vaccine, Benzol, etc.			1-2
	Sarcoma ascites ↓ Abdomen	Placental extract medical compound (Thmenine)			1 of the 10 cases was negative
	Sarcoma ascites ↓ Testis	Estron medical compound			2 of the 7 cases were negative

Observations on Cell Changes

As preparation, a subcutaneous tumor produced by the injection of ascites of Yoshida sarcoma in a rat, was extirpated, and retransplantation was performed with 0.2-0.5 cc of the ascites into the abdominal cavity. Five such cases were obtained and each had an interval of 22, 33, 35, 53 or 67 days respectively between the preparation and retransplantation. After the retransplantation the ascites was taken by puncture daily and morphologically observed, staining it fixed and unfixed.

We found the tumor cell in ascites disappeared in a few days and the quantity of ascites decreased gradually. The results in part are summarized in the following table.

Table 2

Kinds of cells \ Days	1		2	3	4	5	6	7
	15 m	3 h						
Tumor cell	42.8	44.8	41.6	3.4	0	0		
	55.4	9.0	0	0	0			
	32.5	16.0	52.0	66.0	—	—	19.5	0
Mononuclear cell	33.4	22.8	42.0	84.8	78.0	79.5		
	32.8	72.5	89.0	91.5	96.1			
	31.5	42.0	33.5	33.0	—	—	77.0	91.5
Granulocyte	15.4	27.0	9.4	2.6	1.5	7.0		
	4.6	17.5	5.5	4.0	2.5			
	32.5	39.5	12.5	0.5	—	—	1.0	2.5
Lymphocyte	7.0	4.4	5.5	6.8	18.0	10.5		
	7.2	2.5	5.5	4.0	0.5			
	2.5	0	1.0	0.5	—	—	1.5	6.0
Mastcell	1.4	1.0	1.0	1.2	1.0	2.0		
	1.2	1.0	0	0.5	0.5			
	1.0	2.5	1.0	0	—	—	0.5	0
Other cells	0.4			1.2	1.5	1.0		

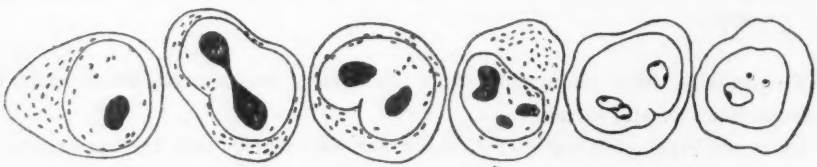
1) Numerals show %
2) The 1st line represents Rat No. 90; 2nd, No. 113; 3rd No. 119 in each cell
3) No. 119 could not be punctured on 4th and 5th days, owing to little quantity of ascites.

To compare these tumor cells with the case of unprepared, we describe the characteristics of the latter.

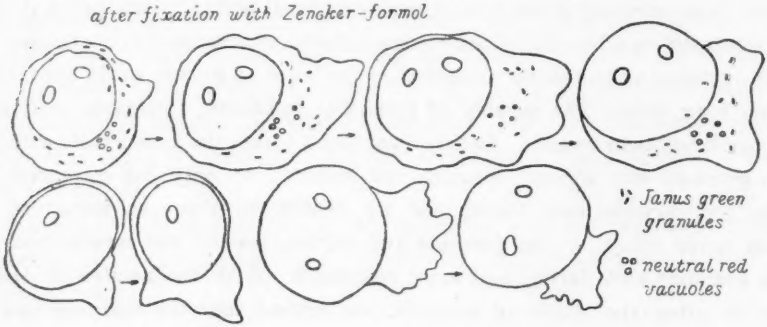
By supra vital staining with Janus green and neutral red, the appearance of neutral red rosette was most notable; and occasionally some other neutral red vacuoles were revealed at various points in the cell body. Janus green granules were scattered, or when the nucleus was omnipresent, crowded in the broader part of the cytoplasma, and they appeared in the form of grains or in short clubs, and no long ones. The number of them was indefinite, sometimes only a few, but sometimes over twenty. Beside these usual types, the tumor cells with Janus green granules but without neutral red rosette, or only 2-3 gathered small neutral red vacuoles were found, and we considered them as immature type. In the early stage of the transplanted ascites, neutral red rosette and Janus green granules were larger and more numerous. With the ascites in the last stage or after the death of animals, we proved that the staining was often impossible. But when the same ascites was once transplanted into new animals, neutral red rosette and Janus green granules revealed immediately—for example, in 15 minutes. These morphological changes, which gave us an impression of the revival of the cell function by the transplantation, present a problem of

the relation between the function or increasing of the cell and the environment. Moreover, if neutral red rosette is closely related with Golgi net, it might give a suggestion on the functional activities of the cell. Otherwise, in the ascites in the later stage, in which Janus green granules and neutral red rosette were revealed, we found the reappearance of these bodies under some other conditions, namely, Janus green granules reappeared distinctly in solution of 0.8% NaCl, 2% $CaCl_2$, 0.4% $MgSO_4$, 0·4% KCl, extremely diluted NaOH, Ringer's, V. B., or 5% glucose, or serum (rat or domestic fowl). Solutions containing Fe, Cu, or Au were not effective, and $NaHPO_4$ and $NaHCO_3$ slightly effective. Compared with the distinctness of Janus green granules, neutral red rosette was uncertain. With staining of Heidenhain's or acid fuchsin after fixation (Zencker's fluid), shape of the granules appeared similar to Janus green granules but the former were more numerous. Nucleolus was large and had various forms, its number was usually 1 or 2 and sometimes more. With Heidenhain's staining, furrows which run over the wall of nucleolus often appeared; sometimes also dots appeared in the nucleoplasm outside the nucleolus.

The ability of emigration and ingestion of the tumor cell was uncertain, but the tumor cell extended or contracted ciliary, tubercular, or tongue formed



Granules and nucleolus of the tumor cell, Heidenhain's staining after fixation with Zencker-formol



Processes of the tumor cell, supra vital staining

processes slowly by supra vital staining; and fine particle of Indian ink injected into abdominal cavity were attracted to the cell body. Still more tumor cell

often ingested large bodies such as erythrocytes.

In the case of the retransplantation, the mitosis of tumor cell was observed till it disappeared, so it showed an attitude of increasing. Toward the disappearance of the tumor cell the cells containing 2 or more nuclei or the cells of irregular forms increased. Otherwise there appeared degenerative forms, showing vacuolisation, cytolysis and karyorrhexis. These were in respect to individual cells, identical with the unprepared cases. But it was notable that just before and after the disappearance of tumor cell, karyoklasis, strongly degenerated cell, phagocyte and medium sized mononuclear cell increased. Similar changes were shown by supra vital staining with Janus green and neutral red. Neutral red rosette and Janus green granules appeared distinctly and continued for a few days, though in some cases neutral red rosette remained much longer. By staining with Heidenhain's or acid fuchsin, the granules of cytoplasm appeared in the same manner.

Summary

1. For the cultivation of Yoshida sarcoma the medium of chicken plasma and chick embryo juice with the addition of rat ascites is most suitable. Addition of a piece of chick embryo heart is also excellent.

2. Other than rat ascites, spleen extracts from normal rats act as growth promoter. Spleen extracts from tumor bearing rats are less effective. Extracts prepared from brain, testis, liver, etc., do not permit the growth of the sarcoma.

3. Tumor reimplanted into the peritoneal cavity, after the removal of the tumor previously successfully implanted in the same rats subcutaneously, resulted in negative, demonstrating the development of immunity. The re-implanted sarcoma cells in these experiments survived generally 2 or 3 days but never more than 6 days in the peritoneal cavity.

4. Injections of tris-β-chloroethylamine into 7 cases resulted in the disappearance of sarcoma cells within 2 days after the first injection. However, due to the strong secondary action no marked prolongation of life was obtained. The suitable doses of nitrogen mustard may be 0.1mg or less per 100g body weight.

5. In 10 cases treated with placenta preparation there was 1 in which the tumor regressed, and in 1 other there was the prolongation of life to 60 days.

6. Estrin was injected subcutaneously into 7 cases in which sarcoma was successfully implanted into testis. Tumor disappeared in 2 of these cases.

7. The tumor was not influenced by mixed typhoid-paratyphoid vaccine, benzene, etc. Placenta preparation and estrin certainly produced regression of tumors in some cases, but the results were not constant.

8. The regressive changes found in sarcoma cells intraperitonelly injected

into animals which were previously successfuly implanted subcutaneously and from which the resulting tumor removed, were identical with those frequently met with in the terminal stage of the usual positive transplantation in normal rats, and there was nothing characteristic about the sarcoma cell changes in immunized animals.

9. In the case of retransplantation in the peritoneal cavity, after the removal of previously implanted subcutaneous sarcoma, granulocytic reaction disappeared in 1 or 2 days, followed by the rapid reciprocal increase of mononuclear cells, in which stage vacuolization of mononuclear cells, appearance of phagocytic cells and desquamation of the lining cells of peritoneum were conspicuous.

10. Sarcoma cells show neutral red rosettes, surrounded by rod-shaped Janus green stained bodies. In some cells the Janus green stained bodies alone, without the neutral red rosette, are seen probably representing younger forms.

11. The Janus green and neutral red stained bodies and granules appear most markedly during 2-3 days after transplantation, and they tend to be less numerous during the middle to terminal stages.

12. Cells not showing stained granules with Janus green-neutral red double supravital technique, if transplanted to a new host, will suddenly show these stained structures, which may be regarded to have some relation to the changes in cellular function or in growth environments.

13. Granules in the sarcoma cells in fixed specimens stained with acid fuchsin or iron hematoxylin are of rod or granular form, scattered in the cytoplasm. Nucleoli are large, of complex form, and occur in irregular numbers.

14. When sarcoma cells do not show stained granules with Janusg reen-neutral red double staining, the addition of certain solutions, such as physiological saline, Ringer's, $CaCl_2$, $MgSO_4$ or KCl in certin concentration, vitamin B_1, glucose, saliva, chicken serum, etc., induces the appearance of Janus green stained granules.

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要　旨

吉田肉腫に対する発育抑制実験

飛岡元彦・上岡和夫
名古屋大学環境医学研究所（所長　勝沼精蔵）

1）本腫瘍の培養には鶏血漿，鶏胎児壓搾液の他大黒鼠腹水を添加した培養基がその發育に最も好適であり，更に鶏胎心臓組織の並置培養も好適である。

2）本腫瘍の培養に於て發育促進物質として腹水の他には正常大黒鼠の脾臓抽出液であり，腫瘍移植大黒鼠の脾臓抽出液はそれより遙かに發育開始の時期が遲れ，且つ比較成長價が低い。之は本腫瘍移植により動物が免疫され，同動物に本腫瘍發育の抑制因子が獲得され，その脾臓にも之が含有された結果であると思われる。大腦，睾丸，肝臓等其他の諸種臓器抽出液添加培地では腫瘍の發育を見ない。

3）本腫瘍を皮下に移植し，一程度增殖後之を摘出し，腹腔内に再移植し陰性の結果を得たことから本腫瘍には免疫が形成されることを證明した。再移植後腹腔内での腫瘍細胞の生存日數は極めて短時日で概ね2～3日で多くとも6日以内である。

4）7例の腫瘍移植大黒鼠に tris-β-chloroethylamine を注射したが初回注射後腹水中の腫瘍細胞は2日以内に消滅した。Nitrogen Mustard では本腫瘍細胞を崩壞消滅させることは出來るが副作用が大で個體も死に至らしめるが，生命の延長は可能で，その量は 0.1mg/100g 以内と考えられる。

5）胎盤製劑 (Thmenin) 投與の 10 例中 1 例に腫瘍の陰性化を見，1 例に 60 日間の長期に亘り生命を維持するのを見た。

6）Estrin 製劑を睾丸内移植腫瘍動物に注射した 7 例中 2 例に陰性化を見た。

7）腸チフス，パラチフス混合ワクチン，ベンゾール等では本腫瘍は影響を受けないが胎盤製劑，Estrin 製劑では影響を受け，確實に腫瘍の陰性化を見る場合もあるが，常に一定した成績を得ることは出來ず，その率は少かつた。以上諸種の藥劑では腫瘍細胞に最も強い影響を及ぼすのは tris-β-chloroethylamine であり，毎常大部分の腫瘍細胞が强烈な潰滅狀態に陷るが，副作用殊に腸粘膜の障害による下痢が强く，比較的早期に死に至り，一定の非常に少量を與えた時にのみ生命の延長を得られるが完全に回復したものはなかつた。

8）皮下移植後腫瘍を摘出してから腹腔内に再移植し陰性化する場合，腫瘍細胞の核萎縮，崩壞，空胞變性等 Giemsa 染色所見は正常の未處置動物に移植した場合の中一末期に屢々見られる所見と同樣で細胞個々に關しては檢索した範圍内では免疫動物に特有の所見とは云い難い。

9) 皮下移植腫瘍摘出後，腹腔内に再移植する場合，再移植後1~2日で顆粒球の反應が消失し，次に單核細胞が急激に相對的の增殖を示す。この際單核細胞の空胞變性，貪喰細胞の强い出現，時に腹腔内固定細胞の剝離等が目立つ事がある。

10) 腫瘍細胞はヤーヌス綠，中性赤複染色により中性赤ロゼツテを見，之を取圍む短桿狀ヤーヌス綠可染體を見る。尙ヤーヌス綠可染體だけで中性赤ロゼツテを見ない幼若型と考えられる細胞を見る。

11) ヤーヌス綠，中性赤複染色による可染體及び顆粒は移植後 2~3 日内に最も顯出がよく，中一末期ではその現われる數が減少する傾向がある。

12) ヤーヌス綠，中性赤超生體複染色による可染體及び顆粒の顯出しない場合でも，之を新しい動物に移植する時は急激に顯出する。之は細胞の機能或は增殖と環境の變化に關係する問題と考えられる。

13) 固定標本に於て酸フクシン或は鐵ヘマトキシリンを用い腫瘍細胞に檢出される顆粒を見ると短桿狀或は顆粒狀を呈し，胞體に散在する。核小體は大型複雜な形態を示し數も一定しない。

14) ヤーヌス綠，中性赤複染色で顯出しない場合でも 2, 3 の溶液例えば生理的食鹽水，Ringer 氏液，一定濃度の $CaCl_2$, $MgSO_4$, KCl 溶液，Vitamin B, 葡萄糖，唾液，血清（鷄）等でヤーヌス綠可染體が顯出する。

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PURIFICATION OF TOXOHORMONE. A SECOND STUDY ON TOXOHORMONE, A CHARACTERISTIC TOXIC SUBSTANCE PRODUCED BY CANCER TISSUES

WARO NAKAHAKA and FUMIKO FUKUOKA

Cancer Institute (Japanese Foundation for Cancer Research) and
Scientific Research Institute, Tokyo

INTRODUCTION

Biochemical studies, especially those by Greenstein [1], on the systemic changes in tumor bearing animals strongly suggested that cancer tissues may produce some toxic substance, the action of which is to bring about a marked reduction in the catalase activity of the liver, which is probably the most characteristic of the changes found in association with neoplastic growth. It seems fair to state, however, that the existence of the toxic substance in question was not demonstrated until 1948, when we succeeded in separating it from a variety of human malignant tumors in crude, yet sufficiently concentrated state to be biologically assayable (Nakahara and Fukuoka [2]). It was then that the designation of "toxohormone" was proposed for the substance.

Toxohormone is the pathological counterpart of hormones. It is produced by malignant tumor tissues, whence it reaches the liver via the blood stream, and deleteriously affects the enzymatic system of that organ. The term "cancer toxin" was avoided, partly to distinguish our substance from all the hitherto alleged cancer toxins, but more especially because of our belief that the term toxin should be restricted to exogenous toxic substances. Cancer tissue, being a part of the animal body, its product is endogenous, and hence is similar to hormones, except that its action is deleterious to the body physiology.

The chemical nature of toxohormone was not satisfactorily elucidated. The method of preparing our crude concentrate indicated that the substance is thermostable, water soluble, noncoagulable by heating, and is precipitable from the watery solution by two volumes of absolute alcohol. It was also demonstrated that the substance is not associated with nucleoprotein or polysaccharide fraction. The concentrates obtained were active for mice in 50–100mg doses.

Since the publication of our previous papers, we made further attempts at the purification of toxohormone, and, so far, we have succeeded in obtaining fractions active for mice in 5mg amounts. The purpose of the present paper is to describe our experiments leading to these results.

Materials

The starting meterials for the purification experiments to be reported here were, for the most part, pooled samples of the alcoholic precipitates, each tested and proved to be active in 50-100 mg doses. These were separated from human cancer tissues, mostly gastric and rectal carcinomas, by our previous method as follows: Cancer tissues, either fresh or in the form of dried material, were extracted with distilled water under heating in a boiling water bath for an hour or two, the watery extract evaporated to a suitable volume and precipitated by adding twice the volume of absolute alcohol. The precipitates were dried and washed with ether. In addition to these pooled samples, we also used alcoholic precipitate separated by the same method from a reticulo-sarcoma. This precipitate was previously proved to be active in 100mg doses.

For control, a pooled sample of alcoholic precipitates prepared by the same method from normal gastric or intestinal mucous membrane was used.

Methods for Assaying the Toxohormone Activity

The toxohormone activity of the samples to be tested was assayed by the amount necessary to produce the depression of the liver catalase activity beyond the lowest limit of the normal variability.

For this purpose samples in the form of watery solution were injected intraperitoneally into young normal mice of 10-15 g body weight, and 20-22 hours after the injection the mice were killed by exsanguination and the liver removed. 0.1 g of liver was thoroughly ground in a mortar, extracted with 10cc of phosphate buffer solution of pH 7, and centrifuged at 3,000 r. p. m. for 5 minutes. 0.3cc of the extract so obtained was made up to 1cc with the same buffer solution, and its catalase activity was measured gas-volumetrically by means of a Battelli-Stern apparatus at room temperature. The cc of oxygen obtained after the reaction with 10cc of 3 per cent solution of hydrogen peroxide for 5 minutes was recalculated to that at the standard condition. We continued to use this simple, although somewhat primitive method, not only as most convenient but also as quite adequate for our purpose. We have reasons to believe that titration with potassium permanganate will yield entirely comparable results.

A considerable individual variation exists in the catalase activity of normal mouse liver as determined by the above method. We have on hand data for 84 normal mice, including all the cases carried as blank controls to the various tests we made. Expressed in the term of the cc of oxygen, these data indicate the maximum and minimum normal activities to be 16.4 (exceptional) and 4.6,

respectively, the average being 7.85.

In no normal mouse was the catalase activity value less than 4 cc, and anything below 5cc was so rarely seen to be regarded as significant in the study of toxohormone effect. These data served as standards in our toxohormone assay. See Chart 1, appended at the end of the descriptions of purification experiment.

Purification Experiments

Precipitation with Trichloroacetic acid

On a previous occasion we separated a polysaccharide fraction from a pooled cancer tissue dessiccate and found it to be devoid of toxohormone activity. The method used of preparing this fraction was that of Boivin, frequently employed for obtaining bacterial polysaccharide, and it involved the deproteinization of watery extract with trichloroacetic acid and precipitation from dialysed solution with acetone. It was pointed out at that time that toxohormone may be inactivated or precipitated by trichloroacetic acid, or it may be dialysable.

The following experiments demonstrate that trichloroacetic acid effectively precipitates toxohormone:

500 mg of pooled alcoholic precipitate was dissolved in distilled water by heating in steam bath, and the small amount of insoluble matter was centrifuged off. To the supernatant was added equal volume of 10 percent solution of trichloroacetic acid, thereby producing whitish precipitate, which was then dissolved in distilled water with aid of a little NaOH, and was re-precipitated by adding two volumes of absolute alcohol, the whole being acidified to pH 3.5 with acetic acid. The yield of the final precipitate: 70 mg.

This precipitate was found to be active in 25mg amounts:

Mouse No.	Amount injected mg.	Liver catalase activity. Oxygen cc
1	25	4.4
2	25	2.8
3	0	10.2

The above experiment was repeated with another pooled sample of alcoholic precipitate. Under the identical treatments 4 g of this sample yielded 550 mg of purified preparation, which was also found to be active in 25 mg amount:

Mouse No.	Amount injected mg.	Liver catalase activity. Oxygen cc
1	25	4.2
2	25	3.7
3	0	9.7

Precipitation with Picric Acid

500 mg of pooled alcoholic precipitate was dissolved in distilled water in a steam bath, insoluble matter being removed by centrifugation. To the solution was added saturated solution of picric acid drop by drop at the rate of 2 drops per 1cc. No precipitate was produced, showing that protamine was not contained in appreciable amount. Acetic acid was then added to the picrinated solution, whereupon yellowish precipitate resulted. This precipitate was washed with acetone three times, dissolved in distilled water with a little NaOH, and re-precipitated with two volumes of absolute alcohol with a little acetic acid. The yield of the precipitate: 100 mg.

This precipitate proved to be active in 25mg doses:

Mouse No.	Amount injected mg.	Liver catalase activity. Oxygen cc
1	25	3.3
2	25	3.0
3	0	8.2

Fractionation with Ammonium Sulphate

1 g of pooled alcoholic precipitate was dissolved in distilled water in a steam bath, insoluble matter, amounting to 100 mg, was centrifuged off, and the solution was brought to $^1/_2$ saturation, and then to the full saturation with ammonium sulphate, the precipitates produced being separated each time.

The two precipitates and solution (peptone fraction) were thoroughly dialyzed against cold running water.

Yields: Precipitate at $^1/_2$ saturation, 250 mg. Precipitate at full saturation, 300 mg. Peptone fraction, 50 mg.

These fractions, as well as the insoluble matter set aside at the outset, were tested as to their toxohormone activity with the following results:

Fraction	Mouse No.	Amount injected mg.	Liver catalase activity Oxygen cc
$^1/_2$ sat.	1	25	3.8
	2	25	3.4
Full sat.	3	25	3.3
	4	25	4.7
Peptone	5	50	Died overnight
Insoluble matter	6	100	5.9
	7	100	5.1
	8	0	5.2
	9	0	6.9

Purification with Cupric Sulphate and Hydrochloric Acid

In the first experiment alcoholic precipitate was reprecipitated with trichloroacetic acid before treating it with cupric sulphate.

1 g of pooled alcoholic precipitate was dissolved in distilled water and treated with trichloroacetic acid as before, yielding 300mg of precipitate. 100mg of this precipitate was dissolved in distilled water and cupric sulphate added to 1 percent, producing voluminous blue-green precipitate, which was washed twice with distilled water and then with N/10 HCl. This caused a pure white precipitate to separate out, which was washed with N/10 HCl and then with distilled water, after which the precipitate was dried and washed with ether. Yield: 40 mg.

This precipitate was found to be active in 5mg doses:

Mouse No.	Amount injected mg	Liver catalase activity. Oxygen cc
1	15	3.7
2	15	3.4
3	5	3.5
4	5	3.4
5	0	5.4

In the above experiment it was suspected that the whole of trichloroacetic acid precipitate was precipitated by cupric sulphate and hence the treatment with the latter unnecessary. We next treated trichloroacetic acid precipitate directly with N/10 HCl.

100 mg of the same sample of trichloroacetic acid precipitate used before was extracted with 20 cc N/10 HCl at room temperature. Precipitate was separated by centrifugation, rapidly washed with distilled water, dried, and washed with ether. Yield: 42 mg.

Test showed that this precipitate was also active in 5mg doses:

Mouse No.	Amount injected mg	Liver catalase activity. Oxygen cc
1	5	2.8
2	5	2.7
3	0	6.4

In the third experiment, the use of trichloroacetic acid was omitted, treating alcoholic precipitate with cupric sulphate.

500 mg of pooled sample of alcoholic precipitate was dissolved in distilled water under heating. Insoluble matter was removed, and to the solution $CuSO_4$ was added to make 1 percent. The bluish precipitate produced was washed with distilled water and then treated with N/10 HCl. The white precipitate obtained

was washed with N/10 HCl, then rapidly with distilled water, dried, and washed with ether. Yield: 75 mg.

This precipitates was active in 5mg amounts, and also in 2.5mg doses:

Mouse No.	Amount injected mg	Liver catalase activity. Oxygen cc
1	5	2.7
2	5	2.7
3	2.5	2.5
4	2.5	4.3
5	0	5.2

An experiment similar to the above was repeated, using, however, alcoholic precipitate prepared from a reticulo-sarcoma tissue.

2 g of the reticulo-sarcoma alcoholic precipitate, tested and found to be active in 100 mg doses, was dissolved in distilled water, and $CuSO_4$ solution was added so as to make 1 percent. The precipitate was treated with N/10 HCl as usual, yielding 170 mg of the final precipitate.

The precipittate was active in 5mg amounts:

Mouse No.	Amount injected mg	Liver catalase activity. Oxygen cc
1	5	3.0
2	5	3.3
3	5	4.0
4	5	4.4
5	0	10.9

Normal Tissue Control

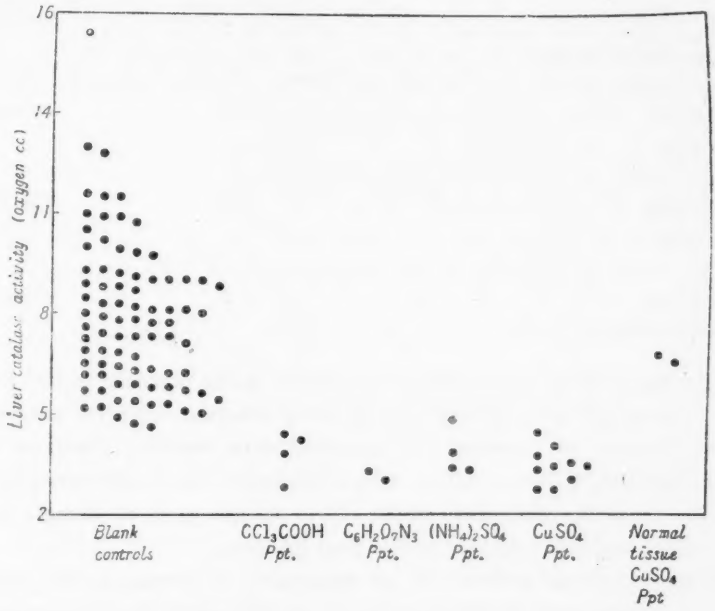
It was previously demonstrated that alcoholic precipitates prepared from normal tissues are without the toxohormone activity when tested in 100 mg doses. Now that we found an efficient method for the purification of toxohormone from cancer tissues, it became necessary to apply the same method on normal tissue to see if the latter would not yield an active fraction.

800 mg of pooled alcoholic precipitate from normal tissues (gastric and intestinal mucous membranes from patients with gastric cancer or with gastric ulcer) was dissolved in distilled water, and $CuSO_4$ added to 1 percent. Precipitate produced was treated with N/10 HCl, washed with distilled water, dried and washed with ether, exactly as in the preceding experiments. The yield of the final precipitate was 170 mg.

The result of the test, as shown below, indicated that toxohormone was not obtained from normal tissues by the method which yields highly active purified toxohormone from malignant tissues.

Mouse No.	Amount injected mg	Liver catalase activity. Oxygen cc
1	50	Died overnight
2	50	6.5
3	50	6.7
4	0	8.7
5	0	5.7

Chart 1. Toxohormone effect of various cancer tissue fractions, contrasted with blank and normal tissue controls.

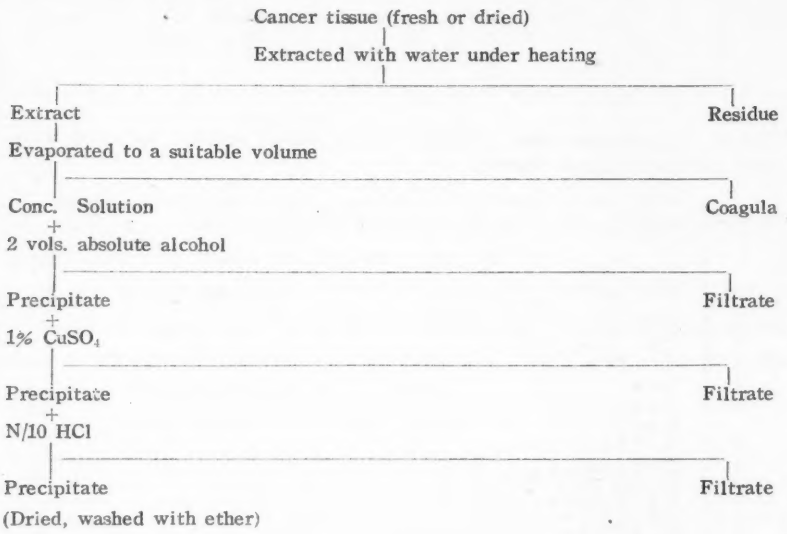


Discussion

The principal steps in the purification of toxohormone as carried out by us consist of (1) extraction with water under heating, removing heat-coagulable matter, (2) precipitation with two volumes of alcohol, (3) precipitation with a protein precipitant, (4) removal of substances soluble in N/10 HCl, and finally (5) removal of ether soluble matter. By these processes toxohormone was purified to the point where it is active in 5 mg doses for mice. The yields of the purified fraction may be roughly stated to vary from 300 to 500 mg per 100 g of dried cancer tissue.

The protein precipitant to be used may be trichloroacetic acid, picric acid,

or ammonium sulphate, but, in view of the subsequent use of N/10 hydrochloric acid, cupric sulphate may conveniently be chosen. The following standard method may be suggested for the purification of toxohormone:



The purified toxohormone fraction is greyish white powder, rather light in weight. It gives all the ordinary protein color reactions with the exception of Molisch's. As may be expected the xanthoprotein reaction gives no yellow precipitate but only yellow solution. As to Molisch's reaction it may be pointed out that the alcoholic precipitate in the above scheme gives a strongly positive reaction, which become negative in the final product.

According to an old scheme for the separation of certain of the proteoses, the purified toxohormone fraction on hand may be said to have the character of the primary rather than of the secondary proteose: Its solution is not coagulated on heating; it is precipitated by half saturation with ammonium sulphate, and it also gives a precipitate in the cold with cupric sulphate. That toxohormone is precipitated by two volumes of strong alcohol shows that it may be close to the so-called "hetero-proteose". Needless to say, however, that these characteristics give no indication as to the relative size of the toxohormone molecule or its complexity. In all probability toxohormone may be a polypeptide, and if so, future investigations on the chemistry of toxohormone face the difficult task of the isolation and identification of the specific polypeptide.

Conclusion

Starting with pooled samples of the crude fraction in the form of alcoholic precipitates, tested and proved to be active in 50-100 mg doses, we succeeded in purifying toxohormone to the point where it is active in 5 mg amounts. The method employed in this purification was to separate the protein fraction by means of a precipitant and to remove from it substances soluble in N/10 hydrochloric acid. Toxohormone may be a kind of polypeptide.

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要　旨

Toxohormone の精製

中原和郎・福岡文子
（癌研究所・科学研究所）

我々は癌組織から生産（分泌）され，血流に運ばれ，肝臓に達しその細胞機能（カタラーゼ作用）に障害を與える物質を捕捉してこれを Toxohormone と假稱した。肝臓カタラーゼの顯著な減少は癌に於ける全身的障害の最たるもので (Greenstein) トキソホルモンはその原因物質である。

トキソホルモンの化學的性狀に就いては，前報告に於てそれが耐熱性であり，加熱によつて凝固せず，エーテルに不溶，水によく溶け，水溶液から二倍量のアルコールで沈澱することを述べた。多糖類として或は核蛋白區劃として分離することは出來なかつた。

その後既に報告したアルコール沈澱物 (50～100 mg で有效な標準濃縮物) から出發して，實驗を重ねた結果，その水溶液から硫酸銅で沈澱せしめ，それを 10/N 鹽酸で處理してすこぶる强力な標品を得ることに成功した。

この精製トキソホルモンは 5 mg で充分に有效である。すべての點を綜合してトキソホルモンは一種のポリペプチードであると思われる。

（文部省科學研究費による）。

[GANN, Vol. 41; March, 1950]

PRELIMINARY NOTE ON THE SPECIFIC GRAVITY OF LIVER DURING THE COURSE OF LIVER CANCER PRODUCTION BY BUTTER YELLOW FEEDING*

KAZUO MORI AND SEIKO MOMOKI

(From the Laboratory of Medical Zoology, Showa Medical School, Tokyo)

Since the discovery of the carcinogenic action of o-Aminoazotoluol by Sasaki and Yoshida[1], and of p-dimethylaminoazobenzene (Butter Yellow) by Kinoshita,[2] the behavior of the rat liver in the process of liver cancer production was studied by many investigators from the biochemical stand point.

In this paper, it is reported that the specific gravity of the liver is definitely reduced in the course of liver cancer production by butter yellow feeding.

Experiment and Result

For the determination of the specific gravity copper sulphate method[3][4] was used. The rat was killed by exsanguination and very small slices, just half the size of a rice grain, were cut from the liver. And then each slice of liver was inserted below the surface of the solutions with graded specific gravity, namely, a series of copper sulphate solutions in quantities of 100 cc varying in specific gravity from 1.050 to 1.085.

A large number of female albino rats were maintained on the usual butter yellow rice diet (0.6 g butter yellow in cod liver oil per 1 kg of polished rice) so as to produce liver cancer.[5] At various periods in the course of the feeding a suitable number of the rats were taken at random and the specific gravity of the liver was determined according to the copper sulphate method.

The specific gravity of the liver tissue sample was estimated by the point where it became suspended in the middle layer of a solution of which specific gravity is already calculated by the standardized hydrometer.

As may be seen from Table 1, which shows the result of this experiment, there was a gradual reduction of the average specific gravity of the liver in the process of liver cancer production. It may be noted that the range of variation in the specific gravity of the liver of normal female rat may be represented by 1.075 to 1.080, average being 1.077 as determined by the method used.

On the other hand, the average specific gravities of the slices of liver with uneven surface, liver cirrhosis and cancer were 1.070, 1.067 and 1.059 respectively.

* Aided by a Scientific Research Encouragement Grant from the Department of Education.

The wide range of the individual variation in the value of the change in specific gravity of the liver may coincide with the variation in the susceptibility of individual rats.

Chart 1 shows the average changes in the specific gravity of liver during the course of the production of liver cancer. That the production of liver cancer is associated with the decrease of the specific gravity will be clearly demonstrated.

It is reported by Nakahara and Fukuoka[6] already that the catalase activity of the liver is decreased in the process of liver cancer production by butter yellow feeding. It is noteworthy that the same tendency to decrease is now demonstrated in the specific gravity of the liver of the rats fed butter yellow notwithstanding that there is no obvious relation between catalase content and specific gravity.

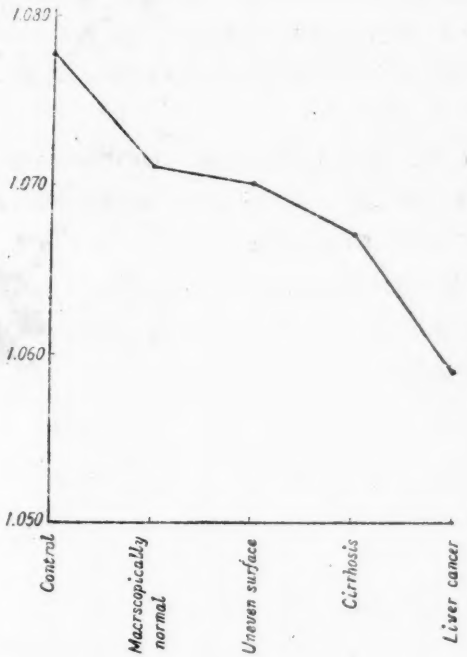
As the method of determination of the specific gravity is so simple and does not take much time in the procedure we expect to carry out an extensive experiment in this direction.

Table 1. Specific Gravity of the Liver at various Periods during Butter Yellow Feeding

Feeding Days	0	20–50	75	100–140	143–168
Liver Findings	Controls	Macroscopically normal	Uneven surface	Liver cirrohosis	Liver cancer
	1.080				
	1.078	1.079			
	1.077				
	1.076		1.076	1.072	
Specific	1.076			1.072	
				1.071	
Gravity		1.070	1.070	1.070	
			1.069	1.068	
			1.067	1.067	1.067
		1.065		1.066	1.064
				1.063	1.064
					1.062
					1.059
					1.059

					1.058
					1.057
					1.055
					1.053
Average	1.077	1.071	1.070	1.067	1.059

Chart 1. The Average Changes in the Specific Gravity of the Liver in the course of the Production of Butter Yellow Liver Cancer,



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要　旨

肝癌生成過程における肝比重（豫報）

森　和雄，百木せい子

（昭和医科大学　医動物学教室）

本下法に従い，Butter Yellow 添加白米で白鼠を飼養し，肝癌生成の實驗過程に於ける肝の比重の變化を追究した。

比重の測定には，近年血清蛋白を測る方法として用いられている硫酸銅法（吉川による）を應用した。卽ち，血清の場合よりは濃度の高い溶液を連續的に調製し，夫々の比重は比重計を用いて決定した。

對照として測定した正常雌鼠の肝の平均比重は 1.077 であつたに反し，實驗經過に應じて分けられた肝所見卽ち肉眼的正常，表面不平滑，肝硬變並に肝癌の 4 期に於ける肝比重は夫々 1.071, 1.070, 1.067 並に 1.059 であつた。

卽ち，肝癌生成過程に於ける肝比重は日を追うて下降している。中原並に福岡によれば，肝癌生成過程に應じて肝カタラーゼが減弱して行く事が明かにされているが，肝比重も同樣の傾向を示す事は注目に値すると思う。

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